

PHARMACOLOGICAL REVIEWS

A quarterly publication in pharmacology, experimental therapeutics, and chemotherapy published for the AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INC., under the supervision of the Board of Publication Trustees: M. H. SEEVERS, *Chairman* MCKEEN CATTELL, L. S. GOODMAN, H. B. VAN DYKE AND ARNOLD D. WELCH.

The Editorial supervision of Pharmacological Reviews is shared jointly with the BRITISH PHARMACOLOGICAL SOCIETY and the SCANDINAVIAN PHARMACOLOGICAL SOCIETY.

EDITORIAL BOARD

L. S. GOODMAN—Salt Lake City, *Chairman*

J. H. BURN—Oxford

J. H. GADDUM—Edinburgh

THOMAS C. BUTLER—Chapel Hill

ALFRED GILMAN—New York, N. Y.

HARRY EAGLE—Washington, D. C.

ERIK JACOBSEN—Copenhagen

U. S. v. EULER—Stockholm

OTTO KRAYE—Boston

MILES WEATHERALL—London

CONTENTS

Hepatotoxic Agents: Mechanism of Action and Dietary Interrelationship by VICTOR A. DRILL	1
Experimental Cardiac Arrhythmias and Quinidine-like Drugs by G. S. DAWES ..	43
The Dosage Schedule of Chemotherapeutic Agents by E. K. MARSHALL, Jr. . .	85

Published by

THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, MD., U. S. A.

PHARMACOLOGICAL REVIEWS

Issued four times a year: March, June, September, and December. One volume of approximately 480 pages per year, subscription price is \$6.00 per volume. For countries outside the postal union add \$.50 per volume. Single copy price \$2.00. Correspondence regarding subscriptions should be addressed to the publishers, or their agents.

Claims for copies lost in the mails must be received within 30 days (domestic). Changes of address must be received within two weeks of the date of issue.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received a pro-rata refund will be made to the subscriber.

To avoid a break in your series, subscriptions should be renewed promptly. The publishers cannot guarantee to supply back issues on belated renewals.

Journal is not responsible for loss of manuscript in the mail. Authors should retain a copy.



THE WILLIAMS & WILKINS COMPANY

Publishers of Scientific Books and Periodicals

BALTIMORE 2, MARYLAND, U.S.A.

HEPATOTOXIC AGENTS: MECHANISM OF ACTION AND DIETARY INTERRELATIONSHIP

VICTOR A. DRILL

*Department of Physiology and Pharmacology, Wayne University College of Medicine,
Detroit, Michigan*

TABLE OF CONTENTS

	Page
I. Introduction	1
II. Histological effects of hepatotoxic agents	2
A. Acute liver injury	2
B. Chronic liver injury	4
C. Centrilobular liver damage and ischemia	5
D. Miscellaneous	7
III. Dietary factors and liver injury	8
IV. Liver function tests	10
V. Hepatotoxic agents and dietary factors	11
A. Chloroform	11
B. Carbon tetrachloride	15
C. Ethylene dichloride	18
D. Propylene dichloride	20
E. Ethyl alcohol	21
F. Benzene	22
G. Brombenzene, methyl chloride	23
H. Trinitrotoluene	23
I. Pyridine	24
J. Azo compounds	25
K. Phosphorus	25
L. Arsenic	26
M. Selenium	28
VI. Possible mechanisms of dietary effects	29
A. Carbohydrate and liver glycogen	30
B. Fat storage of toxic agents	31
C. SH groups and dietary effects	32
VII. Discussion and Summary	32

I. INTRODUCTION

The hepatotoxic action of a number of substances as exemplified by chloroform, carbon tetrachloride or phosphorus has been known for a relatively long period of time. As early as 1882 it was noted by Rosenbaum that liver injury produced by arsenic or phosphorus resulted in a depletion of liver glycogen. The ultimate development of this work was the use of glucose in the treatment of liver injury in both animals and man. Although changes in liver glycogen *per se* are not now believed to influence hepatic damage, such studies in the early 1900s probably served to direct attention to dietary factors. The relation of diet to the toxicity of drugs had been studied by a number of investigators but it was not until 1914 that Opie and Alford investigated the relationship of diet to the effect of hepatotoxic materials. They, and workers following them, demonstrated that the degree of liver damage produced was increased by high-fat diets. With the diets then in use least liver injury was observed when bread and milk was

fed as compared with a high protein diet in the form of meat. Investigation of dietary effects has continued since that time, the techniques and diets being more refined in accord with the newer nutritional data. The advent of choline and methionine, as studied by Best and co-workers and others, and the process of transmethylation, soon led to further studies on the relationship of these and other supplements to the action of hepatotoxic agents.

Studies in this field have aimed at obtaining greater knowledge regarding the mechanism by which the liver injury is produced and the relationship of these effects to diet. Most workers have also had in mind the ultimate use of diet, choline, methionine or other substances as therapeutic materials of possible value in the treatment of a chemically-induced liver injury. However, the fact that methionine decreases mortality in exposed animals does not necessarily mean that methionine has acted on the liver. Indeed, the increased survival produced may be unrelated either to the concentration of hepatic lipids or to liver histology, but rather to the prevention of injury in other organs such as the kidney or heart, as will be discussed later. Some such reports in the literature have been misinterpreted and are often quoted as showing a beneficial effect of the supplement on the liver. In other cases the experimental diets used are so abnormal that it remains to be shown that the results have any application at all to injury produced in man.

II. HISTOLOGICAL EFFECTS OF HEPATOTOXIC AGENTS

The administration of a variety of substances will produce fatty changes and necrosis in the liver, the extent of the injury varying with the dose and route of administration. If a single minimal effective dose is given zonal fatty changes and necrosis will occur, followed by complete regeneration and recovery of the liver. A large dose or injection into the portal vein will produce a massive hepatic necrosis resulting, if the animal survives, in post-necrotic scarring of the liver. The zonal necrosis or the massive necrosis are both acute in nature. The chronic administration of many substances, initially resulting in zonal injury, will eventually cause a diffuse hepatic fibrosis. In addition to the production of acute or chronic injury a delayed type, as produced by cinchophen, should be mentioned, although its cause is unknown.

The acute injury may also be divided according to the part of the liver lobule that is injured. Substances such as chloroform, carbon tetrachloride, tannic acid or mushroom toxin cause injury in the central part of the lobule, whereas phosphorus, allyl formate or *Proteus vulgaris* endotoxin produce periportal damage. This zonal difference in injury will be discussed later with regard to necrosis and ischemia.

Chloroform and particularly carbon tetrachloride have been studied in greatest detail and the hepatotoxic effects of these substances will be summarized. Other damaging agents will be reviewed chiefly in relation to dietary factors.

A. Acute liver injury

Chloroform. As early as 1850 Casper (38), Langenbeck (150) and Mourlon (192) noted the delayed toxic effects of CHCl_3 in man. However, it was not until 1889

that the first delayed death from the clinical use of CHCl_3 anesthesia was reported in which the postmortem examination showed fatty degeneration of the liver (251). In 1866 Nothnagel first observed that CHCl_3 produced fatty degeneration in the liver of experimental animals (199). Later the production of necrosis was recognized and the effect of CHCl_3 on the liver was extended to other species of animals. It is interesting that the liver of the frog, pigeon and terrapin is markedly resistant to the effects of CHCl_3 (191). During this time the action of CHCl_3 on the cardiac muscle and kidney was also observed. The earlier literature regarding the effects of CHCl_3 in animals and man has been reviewed in a number of papers (22, 137, 260).

A careful study of pathological changes produced by CHCl_3 in the dog was made by Whipple and Sperry (264). They noted that the essential change was extensive necrosis and fatty degeneration of the liver and that six to ten hours were required following anesthesia before necrosis could be observed microscopically. Cirrhosis did not follow extensive central necrosis and repair. The repair occurred rapidly, the liver being normal two to three weeks following injury, even though the hepatic artery of the dog was ligated. The necrosis produced in the liver was centrilobular, although, depending on the degree of exposure to CHCl_3 , the necrosis may also extend to the mid-zone and even to the periphery of the lobule. Similar detailed studies have been performed on rabbits (271).

Carbon tetrachloride. Of the hepatotoxic agents, CCl_4 has been the most extensively studied. Most investigations, including the initial studies made by Hall, have been performed on the dog (29, 39, 50, 93, 111-113, 147, 148, 169, 179, 218, 228, 230, 238). Other studies have been performed on the rat (32, 33, 144), guinea pig (208), rabbit (113, 147), and mouse (79). The chicken is evidently very resistant to the effects of CCl_4 (113) and repeated doses of CCl_4 were without effect on the liver of monkeys (146).

The administration of a single effective dose of CCl_4 rapidly produces fatty changes and a centrilobular necrosis of the liver. The maximal histological change is reached within 24 to 48 hours, the severity of the lesions depending in part on the dose and route of administration of the material.

Careful studies of the effects of CCl_4 were first made by Meyer and Pessoa in the dog (179). They noted that the most severe damage was produced in the liver and kidneys. The hepatic necrosis was similar to that induced by CHCl_3 ; it appeared rapidly and was preceded by fatty degeneration. The severity of the lesion was proportional to the dose, although occasional hypersusceptibility was noticed; it also seemed to vary inversely with the age of the dog. Schultz and Marx (230) administered 0.05 cc. of CCl_4 per kg. to eight dogs and obtained fatty degeneration in four and hepatic necrosis in one. Complete studies on the effects of CCl_4 administered in various doses and by various routes to dogs was reported by Lamson and Gardner and their co-workers (93, 147). They observed that the lesions could be detected in 12 hours and were maximal in 48 hours, and although repair began in three to four days, it still may be incomplete after five weeks.

In the rat, subcutaneous injection of 0.016 cc. to 0.03 cc. of CCl_4 per kg. of

body weight produces minimal lesions. Doses of 0.3 to 1.0 cc. per kg. produce more marked changes, usually not fatal (33). Diffuse congestion is first noted, being followed rapidly by hydropic and fatty degeneration and hepatic necrosis. The degree of degeneration and necrosis is dependent on the dose of CCl_4 .

Although changes are noted quite soon, the peak effect of a single dose of CCl_4 is reached in 24 to 48 hours. At this time an exaggeration of the lobular pattern may be grossly observed. Microscopically a zonal necrosis centrilobular in distribution is present, involving every lobule of the liver. The region about the central vein is chiefly affected, although, depending on the dosage of CCl_4 , the reaction may extend to the periphery of the lobule (32). At the height of the reaction the animals may appear ill but are not clinically jaundiced and ascites is not present. By the fourth or fifth day the animal appears improved and at the seventh day seems entirely normal. During this period necrotic cells are being removed, and repair by means of mitosis is occurring. Residual liver damage may still be present at this time. However, within a period of two weeks the surviving parenchymal cells have completely regenerated, the necrotic cells have been removed, and the liver appears entirely normal (33). Histochemical studies of the mouse liver following a single dose of CCl_4 have also been made (246).

Massive necrosis. The direct injection of CHCl_3 , CCl_4 or chlorinated benzene derivatives into the portal circulation produces a very intense or massive liver necrosis which may result in post-necrotic scarring (34, 93, 147, 228, 264). Cameron *et al.* (34) have termed this response, "toxic infarction". Similar results are obtained if the toxin is injected into the spleen. Cameron noted that the liver must absorb these substances quite readily from the circulation since 0.025 cc. of CCl_4 injected into the jugular or ear vein of the rabbit is usually fatal at once, whereas ten times this amount can be introduced into the portal circulation without producing death. Following portal injection, the first signs of necrosis are observed in an hour, and consist of nuclear pyknosis and swelling of the cells, most frequently at the periphery of the lobule. Progressive stages may be seen and by 24 hours the necrotic areas closely resemble infarction. Liver tissue between the lesion appears normal. Progressive regeneration is noticed at seven and 18 days, and by the end of a month smaller infarcts may have completely disappeared. At 104 days all livers were normal except for a slight thickening of the fibrous capsule.

Summary. Depending on dose and route of administration, CHCl_3 , CCl_4 and other compounds can produce various degrees of damage varying from fatty degeneration and necrosis in the central part of the lobule to massive necrosis throughout the lobule. Such acutely induced liver injury is usually followed by complete repair, except for massive necrosis which may be followed by post-necrotic scarring.

B. Chronic liver injury

Cirrhosis. Cirrhosis of the liver can be produced in various species of animals by the repeated administration of CHCl_3 (81, 200). Lamson and Wing (148) gave CCl_4 to dogs for five to six months, and obtained early cirrhosis. They noted that the cirrhosis was similar whether small doses (3 cc.) or large doses (25 cc.) of

CCl_4 were given. In confirmation of these studies a more advanced cirrhosis was produced, typical of Laennec's cirrhosis (29, 169). It was also observed that in certain cases restoration to normal hepatic architecture may take place even after extensive injury (29). Cirrhosis has also been produced in rats (144), guinea pigs and rabbits (254) and pigs (266). Many earlier papers regarding cirrhosis produced by CHCl_3 , CCl_4 and other chemicals may be found in the review by Moon (188). A method has also been described for following the progression of cirrhosis by taking roentgenograms of the liver after the injection of thorium dioxide (245).

The architectural relationship of the connective tissue trabeculae in cirrhotic livers has been studied and most investigators have described the fibrous tissue as originating in the portal areas. However, Ashburn and associates (10), producing cirrhosis by diet or with CCl_4 and alcohol, showed that fatty deposition, ceroid accumulation, and fibrous trabeculae primarily followed the hepatic veins and their terminal (centrilobular) branches. In cases of marked damage, the trabeculae sometimes coursed by or enclosed a portal area. Thus the connective tissue proliferates in the same areas where damage to the liver takes place. Ferris (81), producing cirrhosis in rabbits by CHCl_3 , also noted that the earliest fibrosis was central, and only later was periportal connective tissue involved.

The studies of Cameron and Karunaratne (33) on the production of cirrhosis in rats are of interest. If, with moderate doses, a recovery period of ten days was allowed between injections, each dose of CCl_4 produced the typical centrilobular necrosis. When the chemical was administered at shorter intervals, a diffuse hepatic fibrosis occurred. If the injections of CCl_4 were stopped at the first sign of fibrosis resolution occurred. If the administration was continued until the fibrosis became more pronounced, then, even on discontinuing the injections the fibrosis continued to develop and eventually produced death. Small doses, sufficient to produce only minimal hydropic and fatty changes, administered twice a week for five months, failed to cause permanent damage. *Thus the development and progression of the fibrosis depend upon both dosage and continued injury to the liver, but the process may be completely reversed if the damaging agent is stopped early.* During such a reversal period the collagen content of the cirrhotic liver may be shown to decrease, either by microscopic examination or by chemical determination (190).

Hepatomas. The repeated oral administration of CCl_4 to the C3H or the A or L strain of mice produces a very high incidence of hepatomas (75, 77). The tumors are well-differentiated and resemble those arising spontaneously or induced by *o*-aminoazotoluene (76). Although there is some correlation between the dose of CCl_4 and the incidence of hepatomas, repeated episodes of liver necrosis and its associated chronic regenerative state are probably not necessary for the induction of the tumors (79).

C. Centrilobular liver damage and ischemia

The reason for the production of necrosis by CCl_4 and other compounds about the central part of the lobule, rather than in the periphery of the liver lobule, has only recently been explained. As the blood carrying CCl_4 reaches the liver

the peripheral cells of the lobule are first exposed and yet survive the higher concentration of CCl_4 , whereas the more central cells become necrotic. Survival of the cell seems to depend on proximity to blood supply, which would indicate that the blood, when it reaches the centrally located cells, has been depleted of some vitally necessary substance. Evidence, accumulated over a period of years, indicates that CCl_4 and CHCl_3 produce parenchymal swelling which constricts the sinusoids of the liver, thus producing ischemia and anoxia with consequent damage to the central cells of the lobule.

In 1925 Loeffler and Nordmann (164) observed that the inhalation of CHCl_3 produced narrowing of the intralobular sinusoids in mice and rats. This change, observed directly by transmitted light, occurred within 15 minutes and persisted for several hours. After ten to 24 hours the sinusoids dilated, but three to ten days were required before blood flow returned completely to normal. Later, by the use of a similar technic, the momentary inhalation of CCl_4 in the anesthetized rat was observed to produce an immediate although transient constriction of the sinusoids (258). When the CCl_4 was inhaled continuously for 30 minutes or injected subcutaneously, severe changes were produced. Eight hours later the hepatic cells were definitely swollen and most of the sinusoids were obliterated. Two to four weeks were required before the intralobular circulation returned to normal. With repeated inhalation, to produce cirrhosis, the vascular changes consist in an early and initial vasoconstriction followed by a prolonged secondary obliteration of the vascular channels, chiefly the venous ones, produced by the swelling and fatty degeneration of the hepatic parenchyma.

Injury induced by CCl_4 also interferes with hepatic distribution of substances such as India ink (8, 94, 125). Normally, India ink injected into the spleen, passes to the liver and fills the portal vessels, intralobular sinusoids and hepatic veins. Two hours after administration of CCl_4 the hepatic cells throughout the lobule are swollen and in a majority of the lobules the India ink fails to reach the central zones, which indicates a marked impairment of circulation. Fine vacuoles are also present in the central zone at this time. At four hours the impairment of the sinusoidal circulation is more pronounced and the fine vacuolization in the central cells has increased. Hydropic degeneration is present by the eighth hour. The sinusoids are still closed but penetration of the India ink occurs in a few irregularly distributed areas. At 12 to 24 hours the central hydropic degeneration and necrosis are severe and most sinusoids are still occluded. It is obvious that the degenerative changes produced by CCl_4 follows the restriction of the intralobular circulation. In contrast, allyl formate and the endotoxin of *Proteus vulgaris*, which produce only periportal necrosis, cause dilatation rather than constriction of the sinusoids (125).

Impairment of blood supply to the liver *per se* will produce lesions resembling those induced by chlorinated hydrocarbons. The changes may be produced by ligation of the portal veins or hepatic artery, or both, in various species of animals (35, 163, 177). The necrosis produced is centrilobular, and occurs after approximately the same time interval noted in rats receiving CCl_4 . Anoxia alone can produce degenerative changes in the central liver cells (170). On closer analy-

sis the first effect of anoxia is a fine vacuolization of parenchymal cells which progresses to hydropic degeneration (252), similar to the early effects of carbon tetrachloride. Thus, it seems likely that the central necrosis produced by CCl_4 is a result of ischemia (and possibly anoxia) of the central cells.

It has also been shown by Goldschmidt *et al.* that chloroform anesthesia decreases the oxygen saturation of the portal and hepatic venous blood, indicating hepatic anoxemia (96). They also observed that oxygen protected against CHCl_3 -induced liver injury, and in similar studies oxygen was found to protect against CCl_4 damage (125). If hyperthyroid animals are exposed to low oxygen tensions liver damage is produced which is similar to that seen after CCl_4 (176), and hyperthyroid animals were shown to be sensitive to the effects of CHCl_3 (174, 175).

As noted above, the inhalation of CHCl_3 or CCl_4 for brief periods can produce practically immediate constriction of the sinusoids. This constriction may occur by a direct effect on the cellular membranes or through a nervous reflex (164). There is some evidence for nervous control over intralobular circulation (103), and although chlorinated hydrocarbons have not been shown to activate this mechanism, such action may take place following momentary exposure. With more prolonged exposure it might be supposed that continued sinusoidal constriction may produce parenchymal swelling. However, parenchymal swelling induced by presumably inert substances such as fat or polyvinyl alcohols (114) may also produce a central necrosis. Such infiltration with fat will cause narrowing of the sinusoids (164). Thus it would seem likely that the chlorinated hydrocarbons, by producing severe parenchymal swelling, can directly decrease circulation and produce changes in the central part of the lobule.

Summary. 1. Large doses of hepatotoxic agents, introduced into the hepatic portal circulation, directly produce hepatic necrosis.

2. In comparison, smaller doses of such agents cause parenchymal swelling which is associated with decreased blood flow through the hepatic sinusoids. The resultant ischemia produces a centrilobular necrosis. Anoxia *per se* will produce a similar necrosis.

D. Miscellaneous

Chlorinated hydrocarbons and young animals. The susceptibility to various hepatotoxic agents varies with the age of the animal. During the first three weeks of life puppies are less susceptible to CHCl_3 anesthesia than adult dogs (263). Graham (100) confirmed the resistance in puppies reported by Whipple and noted that when liver glycogen was decreased by fasting, phlorhizin or normal growth, the animals lost their resistance to CHCl_3 . However, probably more important than decreased liver glycogen is the fact that the newborn animals are markedly resistant to anoxia (80). The administration of CHCl_3 to pregnant dogs shortly before delivery or during labor produces the same degree of liver injury as in normal dogs, but the liver of the fetus is not injured (262).

Following this initial period of resistance puppies then appear to become more susceptible to orally administered CHCl_3 (264). A similar result was obtained

with oral CCl_4 in that puppies one to three months of age were found to be more susceptible than adult dogs (93, 147). Both the mortality and the degree of liver injury was increased in such animals. On the other hand, the growing liver cells of young rats six to 50 grams in weight seemed to be markedly resistant to CCl_4 (33). Evidently species differences also exist that may depend on circulatory relationships or different methods of elimination.

Hepatotoxic agents and liver regeneration. Ligation of the hepatic artery in the dog does not delay the liver repair following exposure to CHCl_3 (264). Partial hepatectomy in normal rats is followed by a rapid regeneration of liver tissue. Mann and co-workers (169) noted that dogs with CCl_4 -induced cirrhosis evidenced very little restoration of the liver after partial hepatectomy. Similar results were obtained in rats, in which species it was found that the removal of $\frac{1}{3}$ to $\frac{2}{3}$ of the liver, when advanced cirrhosis was present, was followed by little or no restoration of the liver. With less advanced cirrhosis some regeneration may be noticed. In the pre-cirrhotic stage partial hepatectomy was followed by complete restoration of the organ.

It has been reported that the liver regenerating after partial hepatectomy is more resistant than usual to CCl_4 (144) and CHCl_3 (7). Although Cameron and Karunaratne (33) were unable to obtain such results, others have confirmed the original reports. In dogs recovered from necrosis induced by uranium nitrate the livers showed a greater resistance to uranium and also to CHCl_3 (167). More recent studies have also demonstrated a resistance or decreased sensitivity of the liver of mice to subsequent doses of CCl_4 , and hepatomas induced by CCl_4 were completely resistant (79).

III. DIETARY FACTORS AND LIVER INJURY

The relationship of diet to the hepatotoxic effects of CHCl_3 , CCl_4 and other substances has been the subject of many studies during the past 35 years. More recent investigations have shown that dietary imbalance *per se* may produce liver injury and that choline and methionine can prevent such damage. Such findings soon led to the study of the effects of choline and methionine on liver injury induced by various toxic agents. Before considering these relationships the relative roles of choline, methionine, cystine and vitamin E in dietary induced hepatic damage will be briefly summarized.

Fatty infiltration and hepatic fibrosis. Prolonged fatty infiltration in the liver will lead to fibrosis. The increase in fat in the liver may be produced by two techniques: a) feeding a diet rich in fat; b) feeding a diet low in fat but also low in protein, so that it is deficient in substances that normally prevent an accumulation of fat. The accumulation of fat in the liver may be prevented by substances termed "lipotropic". In initial studies lecithin was demonstrated to be lipotropic and the active material in lecithin was shown to be choline. However, it was soon shown that lipotropic activity was not limited to choline. Casein and other proteins were also observed to prevent the accumulation of fat in the liver; of the various amino acids then studied only methionine was found to be lipotropic. Du Vigneaud then demonstrated that the methyl groups of methionine may be

used for the synthesis of choline. Thus fatty livers may be prevented by diets high in choline or high in protein. Best and Lucas (20) have reviewed the studies in this field. Under certain conditions, still not clearly defined, other substances such as biotin, inositol, thiamine, riboflavin and pyridoxine may influence fatty livers provided by diets high in fat or cholesterol (173). Caffeine, theobromine and theophylline may be partially demethylated and thus possess lipotropic activity (120).

Massive hepatic necrosis. It was first suggested by Daft and associates (48) and later shown that acute massive necrosis can be produced independently of fatty changes and fibrosis in the liver (127, 130, 131). Animals surviving the acute massive necrosis developed postnecrotic scarring and nodular hyperplasia in the liver. The massive necrosis was produced by diets supplying small amounts of casein or containing yeast as a source of protein. The necrosis was prevented by supplements of cystine (130, 131) or methionine (128). Although methionine can be converted to cystine in the body, and cystine can spare the utilization of methionine, it has been shown that dietary induced necrosis is due to a deficiency of cystine (95). Such necrotic livers show only a slight decrease in methionine content and no change in the amount of cystine (55).

However, not all groups, including the author and his colleagues, were able to produce hepatic necrosis simply by feeding a low protein diet (110, 198, 213). In the meantime Schwarz had shown that a deficiency of vitamin E is also necessary to produce necrosis and that vitamin E would prevent massive necrosis (231, 232), a finding later independently confirmed (1, 2, 104, 129). Thus in order to produce massive hepatic necrosis the diet must be deficient not only in cystine but also in alpha-tocopherol, and adequate supplements of either cystine or alpha-tocopherol will completely prevent this type of liver injury.

Liver extract, vitamin B₁₂, folic acid and choline. It has been shown that liver extract is effective in preventing fatty infiltration of the liver induced by a high fat or low protein diet (60, 109). Of particular interest is the fact that liver extract does not contain sufficient choline or methionine to account for its lipotropic activity, and other supplements fail to prevent liver injury although they contain more choline than liver extract (60). Vitamin B₁₂ concentrate prepared from liver also exerts lipotropic activity in rats fed a high fat diet (68), although the activity varies in different preparations (63). Crystalline vitamin B₁₂ does not prevent hepatic necrosis or fatty infiltration resulting from a high fat diet but it can exert a partial lipotropic effect when a low protein, low fat diet is fed (106, 172). The activity of liver extract and vitamin B₁₂ concentrate is not due to a sparing effect of vitamin B₁₂ on the small amount of choline present (172). A combination of small amounts of crystalline vitamin B₁₂, choline, folic acid or citrovorum factor exerts only a partial lipotropic effect (145), and this does not explain the lipotropic effect of liver extract or vitamin B₁₂ concentrate. Vitamin B₁₂ concentrate has also been reported to inhibit the histological changes and the deposition of liver fat induced by CCl₄ (210).

Inanition, food intake, and response to drugs. In general the toxicity of many, but not all, drugs is increased in the fasted animal. Earlier studies on the effect of

restricted food intake or starvation on the toxicity of many materials have been summarized (133). When agents that can produce injury to the parenchyma of the kidney or the liver are administered, it is usually noted that such damage is increased by fasting. It is thus important in reading and comparing papers to note whether the animal has received food *ad libitum* throughout the study or whether food has been withheld for 24 to 48 hours before the administration of the hepatotoxic agent.

Even though food is not withheld the food intake will vary considerably, particularly during a chronic study. Many hepatotoxic agents produce a decrease in food consumption, thus limiting not only methionine or choline intake, but also important factors such as the other amino acids. This may in turn influence weight gain and mortality, independent of the degree of hepatic damage that is present.

During treatment, measurement of food intake is also important. A therapeutic substance may be effective because it produces an increase in food intake. For instance, thyroid feeding will increase the requirements for various B vitamins, and the feeding of yeast supplements will delay the onset of liver injury in hyperthyroid dogs. The yeast supplements are effective because they increase the food consumption of the animals (61, 69). The relationship of yeast and food intake to the effects of CCl_4 was studied by Post and co-workers (211). Using isocalorically fed rats, they found that large supplements of brewer's yeast partly counteracted the growth-inhibiting effect of CCl_4 but were without effect on the liver injury. In contrast to the yeast intake, the food consumption modified the severity of the cirrhosis. Animals fed eight to 11 grams of food per day had more severe liver lesions than those receiving 14 grams a day.

IV. LIVER FUNCTION TESTS

Various tests of liver function may be used to follow the progress of damage induced by hepatotoxic substances or dietary means. Drill and Ivy (62) made serial studies of such tests in dogs receiving CCl_4 twice a week. The determination of bromsulphalein retention was found to be the first and most consistent test to become abnormal, being closely followed by an elevation in alkaline serum phosphatase. The intravenous galactose tolerance test and changes in prothrombin time (one-stage technic) were not as sensitive as the other tests. Determination of serum bilirubin in the dog was of no value. The study emphasizes the quantitative association of the functional tests rather than a qualitative dissociation.

A comparative study of liver function tests was also made by Svirberly and associates (248) in dogs exposed to xylydine. Again the bromsulphalein test was found to be the most reliable one for detecting liver damage. The Rose Bengal dye retention test was nearly as sensitive, but had certain disadvantages. Next in order of value were the alkaline serum phosphatase and prothrombin determinations. The determination of bilirubin clearance and urinary urobilinogen gave still less consistent results. The van den Bergh reaction and determination of icterus index or fibrinogen were of little value.

This order of efficiency of liver function tests in dogs agrees well with the results obtained in human volunteers with induced hepatitis (57, 197). The dog differs from man, with regard to liver function tests, in that normal dog serum usually does not contain bilirubin. Injected bilirubin is also excreted faster in the dog than in man. The cephalin flocculation test cannot be used for experimental studies as normal dog serum produces flocculation. The thymol turbidity test was also noted to be valueless in detecting CHCl_3 -induced injury in the dog (58).

Changes in dye retention (Rose Bengal) and alkaline serum phosphatase have been studied in protein depleted dogs (159). Irrespective of the diet, all dogs with increased liver lipids or cholesterol showed dye retention and an increased serum phosphatase. However, the degree of functional change was not necessarily proportional to the fattiness or cholesterol content of the liver.

The liver function tests are probably not affected by a decrease of body weight in the dog, provided sufficient lipotropic factors are supplied. Hough *et al.* (134, 135) produced an increase in dye retention and serum phosphatase in dogs fed a low protein diet, which could be prevented by the addition of casein, cooked egg white or choline. The changes in liver function do not seem to be related to a fall in body weight, for the animals receiving no supplements or a daily supplement of choline showed in general a comparable decrease in weight. Miller and co-workers (182) reported similar results, obtaining a decreased bilirubin clearance when the low protein, low choline diet of Hough was fed, but not when the low protein diet was supplemented with choline. In studies on the relationship of a chronic vitamin B complex deficiency and protein intake, Drill and Loomis (66) did not obtain changes in liver function in inanition control dogs receiving adequate yeast, even though body weight decreased.

V. HEPATOTOXIC AGENTS AND DIETARY FACTORS

A. Chloroform

Various substances, other than dietary factors, have at times been reported to protect against the effects of CHCl_3 . Some of these agents are not specific and the effects of others, such as sulfanilamide, are unexplained. Quinine sulfate was earlier observed to exert a marked protective effect against CHCl_3 liver injury in dogs (53). Later Forbes and co-workers described an aqueous extract of hog's liver that prevented hepatic necrosis induced by CHCl_3 (88). The extract was purified, as described later under CCl_4 , and the active material found to be sodium xanthine which also protected against hepatic damage produced by CHCl_3 (196). This action of sodium xanthine was confirmed by Ravdin *et al.* (215). However, they also showed that this effect was not specific for xanthines but could also be obtained with substances such as ricinoleate or colloidal carbon.

Sulfanilamide was also observed to protect rats against CCl_4 -induced damage (cf. CCl_4). Forbes and Evans (84) extended their studies to include observations on the action of sulfanilamide during CHCl_3 inhalation in rats, and again

noticed protection as judged by the degree of hepatic necrosis. Similar findings were obtained in dogs given sulfanilamide for five days and fasted for three days before CHCl_3 anesthesia (250). In sulfanilamide-treated dogs, hypoprotrombinemia and histological changes were less severe than in control dogs. Sulfanilamide given during the anesthesia did not protect against the liver injury induced by CHCl_3 . The possible relation of this action of sulfanilamide to thyroid activity is discussed under CCl_4 .

CHCl_3 and diet. CHCl_3 was the first hepatotoxic substance to be studied in relation to diet. The early observations were made with mixed diets that would not now be considered satisfactory in all respects, and it was not until 1924 that synthetic diets containing known amounts of casein, carbohydrate and fat were used.

Opie and Alford first studied the relation of diet to the hepatotoxic effects of CHCl_3 (201-203). They noted that mortality following CHCl_3 was increased by a high fat diet. Meat feedings increased the survival period but best protection was obtained with a high carbohydrate diet of oats and sugar. However, in all groups animals which died early showed a similar degree of hepatic necrosis. These observations were later confirmed in dogs, a carbohydrate diet giving best protection, a high fat diet no protection, and a meat diet being intermediate (52). The feeding of casein to two dogs also offered protection whereas starvation increased the severity of the liver damage. At the same time Graham (100) also reported that the administration of glucose at various intervals decreased the hepatic injury produced by CHCl_3 . Chloroform also increases nitrogen excretion, and it was found that the elimination of nitrogen and the liver injury following CHCl_3 were greater in a fasting than in a sugar-fed animal (51). Liver repair was also more rapid and complete on a sugar or high carbohydrate diet than during starvation. In a further comparison of diets, best repair of the liver following CHCl_3 exposure was obtained with a diet of bread and skim milk (54). Repair was slower with a diet of lean meat, whereas when fats were fed the rate of regeneration was not influenced and was similar to that observed in fasting animals. These initial studies all showed that protein, in the form of meat, offered less protection against CHCl_3 than a diet high in carbohydrate.

Further studies were reported in 1924 by Moise and Smith (187) who were the first to use diets composed of various proportions of casein, carbohydrate and fat in studying the responses to hepatotoxic agents. With such diets the responses to CHCl_3 were slightly different from those obtained with the earlier diets of meat or bread and milk. They fed a standard diet containing 18 per cent casein and 22 per cent fat. Increasing the fat in the diet produced more extensive hepatic necrosis whereas decreasing the fat content and increasing either the amount of carbohydrate or casein lessened the severity of the liver injury produced by CHCl_3 . Both the high carbohydrate diet and the high protein diet thus offered greater protection than the standard diet. Further comparison between the high protein and the high carbohydrate diet could not be made as the fat content of the diets varied. The results of this study show that a high protein diet in the form of casein gives good protection as compared with the poor protection reported earlier for protein in the form of meat. Moise and

Smith also observed that the rate of repair was greatest with the standard balanced diet. In a further study (239) they noted that the protein gliadin did not offer as much protection as casein, and the substitution of gelatin further increased the degree of liver injury resulting from CHCl_3 . Goldschmidt and co-workers (97) fed six different diets for periods of two to four weeks and found that the incidence and the severity of liver injury 24 hours after CHCl_3 anesthesia, increased progressively with an increase in the concentration of hepatic lipids. It is difficult to compare the effects of high and low protein intakes as the fat content of the diets varied. However, when rats with similar concentrations of hepatic lipids were compared, the total incidence of liver damage following CHCl_3 was not significantly different in high protein and low protein diets. The severity of damage on the low protein diet appeared to be greater, but the comparison was not strict as eight of 17 rats were fed only sucrose for the seven days before CHCl_3 anesthesia. The CHCl_3 anesthesia produced hepatic damage in 10 per cent of the animals fed a stock diet; the only synthetic diet giving a comparable low incidence of injury contained 16 per cent casein and 77.5 per cent sucrose. Diets high in carbohydrate or high in protein decreased the hepatic damage following CHCl_3 but more direct comparison cannot be made as the diets varied in fat content. The above reported studies are all acute in nature; no observations on diet in relation to chronic exposure to CHCl_3 have been reported.

CHCl_3 is very toxic to fasting animals and the injury produced to the liver is equal to or greater than that observed in animals fed a high fat diet (52, 97, 187). If dogs are *markedly* depleted of protein they also become very susceptible to CHCl_3 (184). Such animals were fed a diet *nearly devoid* of protein for four to nine weeks with, in most cases, additional bleeding or plasmaphoresis further to deplete protein stores. Such dogs, fasted for 24 hours before anesthesia, died 15 to 65 hours following a brief period of CHCl_3 anesthesia. It is of interest that even after such depletion a single meat feeding 24 to 48 hours before anesthesia protected against CHCl_3 injury (183, 184).

The degree of susceptibility to CHCl_3 depends a great deal on the severity of the protein depletion. In a further study dogs were fed a moderately low protein diet (eight per cent casein) and a normal (20 per cent casein) diet, both adequate in all other respects, for prolonged periods (58). CHCl_3 was then administered to each group and the changes in bromsulfalein retention, serum bilirubin, alkaline phosphatase and thymol turbidity studied. After nine weeks on the diets, CHCl_3 was administered orally and no difference in response between the two groups was observed. Dogs fed the eight per cent casein diet for 16 weeks and then anesthetized with CHCl_3 for one hour showed a slightly greater retention of bromsulfalein and a slower return of this function to normal, when compared with the 20 per cent casein group. In a further test the low protein diet was fed for 35 weeks, and such dogs showed only a slight degree of sensitivity to oral CHCl_3 . In all cases the only difference between the groups was obtained with the bromsulfalein test; the results of other tests, although they became abnormal, did not differ significantly between the two groups.

Choline and methionine. Supplements of choline did not prevent hepatic necro-

sis following CHCl_3 anesthesia in rats receiving a low protein, high fat diet, or in rats fasted for 24 hours (97). The known effect of CHCl_3 to cause an increased excretion of nitrogen and sulfur (47, 137) and the fact that casein and beef muscle contain methionine and cystine soon led to a trial of these substances for the prevention of liver injury. This problem was first investigated by Miller, Ross and Whipple (183) who showed that methionine, and to a lesser extent cystine, given 24 to five hours before CHCl_3 anesthesia, offered almost complete protection to protein-depleted dogs. The dogs were fed a diet with nearly zero protein content for four to 10 weeks and were fasted 24 hours before the anesthesia. Supplements of other non-sulfur containing amino acids did not protect against the CHCl_3 poisoning. They also observed, in similar protein-depleted animals, that methionine protected against CHCl_3 toxicity when administered three to four hours after anesthesia, but not when given four to six hours later (185). In view of later studies with CCl_4 it is not now known whether the liver, kidney or other organ was the site of action of the methionine. In other studies rats fed a diet moderately deficient in protein (eight per cent casein) and a normal (20 per cent casein) diet were exposed to chronic inhalation of CHCl_3 for a 70-day period. The CHCl_3 caused a decrease in the weight gain of each group, not corrected by methionine. Similarly the hepatic lesions produced by CHCl_3 were not significantly changed by methionine in rats on either diet (59). These results are similar to the lack of effect of methionine on the liver of rats during chronic CCl_4 exposure. It is obvious that the response to CHCl_3 and the effect of methionine will vary tremendously with the degree of protein restriction and perhaps also with the chronicity of the study.

It has also been reported that methionine and sodium thioglycollate protect against liver injury induced by CHCl_3 anesthesia in dogs receiving a normal stock diet (31). Liver injury was estimated by the bromsulfalein test and the data based on CHCl_3 exposures two months apart. Twenty-four hours after the initial CHCl_3 exposure the bromsulfalein retention for each group varied between 22 to 39 per cent. A similar average spread and variation of 17 per cent were present after the second CHCl_3 exposure. Further, the control group and the methionine-treated group differed from each other by about 10 per cent retention of dye, the values still lying within the spread of 22 to 39 per cent retention. With this variability it is difficult to conclude that there is a positive effect of methionine, particularly because each curve is only an average, without the range or the standard error being given. The bromsulfalein test, although sensitive in detecting liver injury, varies considerably with regard to individual animals and on different days, as may be seen in related data on dogs (65, 67).

Clinical studies. Although no clinical studies of methionine or choline therapy during CHCl_3 exposure have been reported it should be pointed out that the best effects of methionine have been obtained in animals fed a diet nearly devoid of protein for many weeks. Such conditions are not likely to be comparable to those seen in man, a fact overlooked by many who have misinterpreted the experimental studies with CHCl_3 and applied the conclusions to other instances of liver injury under different dietary conditions.

Summary. Both a high fat diet and fasting increases the hepatotoxic effect of CHCl_3 . Diets low in fat and rich in carbohydrate and protein give the best resistance to CHCl_3 and allow active regeneration of hepatic cells. Moderately protein-deficient diets only slightly affect the response to CHCl_3 whereas diets nearly free of protein markedly increase susceptibility. Methionine has been the chief supplement studied, and the best effect of this material has been obtained in animals fed a diet nearly devoid of protein for a prolonged period.

B. Carbon tetrachloride

Various substances exist which display a protective effect against CCl_4 -induced injury. The effects, although reproducible, often are not specific, and do not seem to be related to known actions of dietary factors. In 1936 Forbes and co-workers reported the preparation of an aqueous extract of hog's liver that protected against hepatic necrosis induced by CCl_4 in rats (88). The material also prevented cirrhosis following repeated exposures to CCl_4 (83). A crystalline material was then prepared from the extract which was identified as sodium xanthine (87, 196). Either the purified crystals or sodium xanthine offered protection against the hepatic necrosis induced by CCl_4 (14, 82, 136, 196). The protective effect of xanthine does not seem to be related to the concentration of liver lipids (85). However, similar effects were obtained with guanine, hypoxanthine, uric acid, nucleic acid, nucleoside guanosine and pyrimidine uracil (196). In view of related studies with CHCl_3 , in which protective effects were obtained not only with sodium xanthine but also with caffeine, sodium ricinoleate and colloidal carbon (215), the effect of sodium xanthine must certainly be regarded as non-specific, although unexplained. In acute studies with CCl_4 , protection against liver injury was reported with sodium thioglycollate, glutathione, sodium thiomalate, sodium glycollate and sodium malate (30). Theophylline is also reported to decrease mortality following the intraperitoneal injection of CCl_4 in rats receiving a 10 per cent casein diet (136).

Leach and Forbes noted that sulfanilamide protected the liver of rats from CCl_4 (154). In a subsequent study sulfanilamide also retarded the development of hepatic cirrhosis produced by CCl_4 (86). This effect has also been confirmed in studies with CHCl_3 . Leach and Forbes suggested that the activity of sulfanilamide might be related to its known ability to inhibit thyroid function. It is of interest that subsequent studies demonstrated a preventive effect of thiouracil or thyroidectomy on dietary induced liver injury (115, 107). However, the lowering of thyroid activity by thyroidectomy or thiouracil, in experiments with pair-fed controls, failed to prevent the liver injury induced by CCl_4 (9), which suggests that the mechanism of the protective effect of sulfonamide in CCl_4 injury is different from that in dietary induced damage.

CCl_4 and diet. All investigations have shown that diets high in fat increase the acute toxicity of CCl_4 (50, 85, 93, 147, 218). Similar results were obtained with the chronic inhalation of CCl_4 in rats (108). In one study a high fat diet did not increase mortality in dogs receiving CCl_4 , but the diet was fed for only four days (46).

Best protection against liver injury induced by CCl_4 seems to be offered by diets high in carbohydrate. Initially Davis (50) reported less hepatic damage from CCl_4 with a carbohydrate diet. The administration of glucose before the inhalation of CCl_4 was noted to protect the liver of cats but not of dogs (39). In rats, glucose or sucrose was without effect when given before CCl_4 inhalation, but when administered at intervals after exposure prevented the usual increase in liver fat (56). Similar results were obtained in dogs. Treatment for 24 hours did not influence the degree of hepatic necrosis but if continued for two to four days less liver injury was evident. Inanition will also increase susceptibility to CCl_4 . Rats with fatty livers from a low protein, high fat diet, starved for 24 hours, were only slightly more susceptible to CCl_4 than normal animals starved for the same period of time (85). Bollman, in various studies, always obtained best protection with a high carbohydrate diet (26-29). His dogs fed a high protein diet (meat) showed greater histological damage after CCl_4 than those fed a high carbohydrate diet. Similarly, in studies on rats a high carbohydrate diet gave the least evidence of histological damage and the longest period of survival. In a discussion of one paper (28) Soskin states that his unpublished results are in agreement with those of Bollman.

The death of exposed animals may be due to factors unrelated to liver injury. Cutler (46) noted that dogs fed a diet of lean meat were much more susceptible to CCl_4 , as judged by mortality, than animals receiving a mixture of lean meat and dextrin or bread and milk. However, although the mortality differed, he was unable to demonstrate any consistent difference in the amount of liver injury produced by CCl_4 with either diet. It is known that intoxication with CCl_4 and other substances produces an increase in blood guanidine which in turn produces hypoglycemia. It has been shown that the increased toxicity of CCl_4 with a high meat diet is correlated with an increase in blood guanidine followed by a severe and often fatal hypoglycemia (45). In a series of studies (186), it has been repeatedly shown that calcium administration, by counteracting guanidine, will markedly increase the survival of meat-fed animals without affecting the incidence or the severity of the liver injury. Thus the susceptibility of animals fed high protein diets in the form of meat is not necessarily related to the lipotropic activity of the diet.

More recent studies also fail to show that a low casein diet increases the liver injury produced by CCl_4 . With a constant fat intake, a high carbohydrate, low protein diet increased mortality during chronic CCl_4 exposure but did not produce any significant difference in hepatic histology when compared with a high protein, low carbohydrate diet (108). However, when rats receiving low casein and normal casein diets were pair-fed no difference in survival time was found after CCl_4 administration (116). In studies of liver injury in dogs, as measured by the bromsulphalein test and histologically, hepatic damage was greater in animals fed the normal protein diet than in those fed a low protein, high carbohydrate diet for three and one-half or 16 weeks (67). The fat content of both diets was constant and there was no significant difference in food intake between the two groups. In other studies rats were placed on a protein-free diet, 18 per

cent casein diet or 54 per cent casein diet for three days and then injected with CCl_4 (36). Liver injury was least on the protein-free diet. Those fed the 18 per cent casein diet showed the greatest degree of hydropic degeneration and those on the 54 per cent casein diet the greatest deposition of neutral lipids.

Choline, methionine and vitamin E. The effect of methionine has been studied under conditions of high, normal and low protein intakes. Drill and Loomis were unable to obtain any added protection against hepatic injury induced by the oral administration of CCl_4 when methionine was added to a high or normal protein diet (64, 65). The results were similar in dogs receiving CCl_4 for short periods of time or up to 64 days. In order to eliminate the factor of absorption Shaffer *et al.* injected CCl_4 intraperitoneally but also failed to find an effect of methionine on liver function tests, hepatic histology or survival of the dogs (235). They were also unable to find any effect of subacute inhalation of CCl_4 on the nitrogen-sulfur ratio of the livers of rats fed a normal diet (236). Supplements of methionine, choline or cystine were also found to be without effect on the hepatic lesions induced in rats fed a normal diet (30, 234).

Choline supplements do not prevent an increase in hepatic lipids of rats receiving a low protein diet and injected with CCl_4 . On the other hand, the addition of choline increased the rate of removal of excess fat from the liver of the poisoned animals (13). With a low protein diet supplements of methionine during chronic CCl_4 inhalation increased survival but did not influence the liver injury produced (108). The results were similar with either a normal or high amount of fat in the diet. A sex difference in response was also noted, male rats being more susceptible than female rats. The methionine supplements increased survival time not by affecting the liver but by preventing the renal necrosis induced by CCl_4 . Methionine was also without effect on the liver injury produced by CCl_4 in dogs receiving a low casein diet, as judged by functional and pathological changes in the liver (65).

The influence of diet and various supplements on cirrhosis induced by CCl_4 has been studied by Sellers, Lucas and Best (233). When such animals were placed on a low casein diet the hepatic lesions did not improve but, as expected, progressed. However, after treatment for two to three months with the low casein diet plus adequate supplements of choline or methionine the liver appeared grossly normal and it was difficult to find fibrous tissue on microscopical examination. A high protein diet containing naturally occurring methionine produced similar results. Supplements of inositol were without effect. It is obvious that the cirrhosis produced by CCl_4 was still in a reversible stage, as discussed previously (cf. II, B), and a more advanced cirrhosis probably would remain unaffected. The authors conclude, "While the experimental evidence indicates that the presence of the lipotropic agents is essential for the repair of the damaged liver under the conditions of our experiments, it does not suggest that the addition of choline or its precursor, methionine, to diets already containing adequate amounts of these factors will enhance the therapeutic effect..."

It has also been recently reported that the addition of methionine or vita-

min E to a low (10%) casein diet will decrease the mortality from acute toxic doses of CCl_4 (136). Protection was not obtained when the diet contained 22 per cent casein. Studies were not made to determine whether the effect of vitamin E or methionine was on the liver or other organs. However, these findings are of interest, particularly with regard to vitamin E, for as summarized in Part III a deficiency of tocopherol or cystine can produce hepatic necrosis.

Clinical studies. The use of methionine received impetus from the report of Beattie and associates (18) who described its administration, along with casein digest, in a patient who ingested an estimated 30 to 40 cc. of CCl_4 . They concluded that methionine exerted a beneficial effect. However, survival from as much as 118 to 148 cc. of CCl_4 has been reported before the advent of methionine (156), so that it cannot be definitely stated that methionine was of value in the above case. Methionine has also been administered to patients following industrial exposure to CCl_4 (71, 72), but a critical reading of these papers does not reveal any evidence of beneficial effect of methionine in the treatment of liver injury.

Summary. Diets high in fat increase the toxicity of CCl_4 . A diet high in carbohydrate and protein offers the best protection against the effects of CCl_4 . Moderately protein-deficient diets do not increase the hepatotoxic action of CCl_4 , although mortality may be greater due to renal injury. Choline does not prevent the increase of liver fat produced by CCl_4 , but increases its rate of removal from the liver.

Supplements of methionine have not been shown to decrease the liver injury produced by CCl_4 in animals fed a normal diet or a moderately low protein diet, although renal injury occurring on the low protein diet may be prevented. Thus there is no experimental support for the use of methionine to combat liver injury induced by CCl_4 in patients and certainly, despite claims to the contrary, there is no good clinical evidence for such an effect. This does not deny a possible effect of methionine on other organs, or its possible value in liver lesions in the face of very severe protein depletion, which has not as yet been studied. When early cirrhosis is induced in rats by CCl_4 , repair of the liver occurs when a normal diet is fed, but there are no indications for supplemental methionine.

C. Ethylene dichloride (1,2-dichloroethane)

Ethylene dichloride is one of the more toxic chlorinated hydrocarbons. Single inhalation exposure has been reported to produce fatty degeneration in the heart, liver and kidney (153, 195). More complete studies have recently been reported by Heppel and co-workers (118, 119). They noted that pulmonary congestion is a constant finding after inhalation, and that many animals develop adrenal cortical necrosis. Renal and liver damage are also produced but the liver injury is very mild compared to that resulting from acute exposure to carbon tetrachloride or chloroform (119).

Normal diet and protective agents. Various chemical agents were found to protect mice, fed a normal diet, against death from dichloroethane (119). Protection was obtained by the oral administration of *p*-aminobenzoic acid, methionine, sulfanilamide and aniline. In one additional test, methionine was administered

after exposure to a high dose of ethylene dichloride, but failed to decrease the ensuing mortality. These compounds, with the exception of methionine, are without known effects on the liver and the liver injury was so slight the decrease in mortality cannot be due to the prevention of liver injury. Since the compounds were administered 30 to 60 hours before the inhalation of ethylene dichloride they probably did not act by depressing the thyroid gland. It is interesting to note that the compounds, other than methionine, all contain an amino group in a benzene ring, but the mechanism of their protective action is at present unknown.

In a further study in rats fed normal diets containing 15 to 25 per cent casein, good protection against death from the inhalation of ethylene dichloride was obtained by the addition of *l*-cystine, *l*-cysteine, methionine, thiourea or thiouracil to the diet (121). Partial protection was obtained with dithiodipropionic acid or 2-thiobarbituric acid. These compounds all can furnish sulfhydryl groups, either present in the compound, or by reduction to thiol compounds, or by existing in a tautomeric equilibrium with a thiol form. Cysteine was not effective when its SH group was blocked as in *S*-benzyl-*l*-cysteine. Also inactive were cysteic acid, $\text{Na}_2\text{S}_2\text{O}_3$, Na_2SO_4 , Na_2S , taurine, tyrosine, thiolactic acid and thiomalic acid, although NaCNS gave a partial effect. Although *p*-aminobenzoic acid offered protection when given by stomach tube it was without effect when added to the diet. Others have also noted a beneficial effect of thiouracil (270). It is doubtful, however, if all the above effective compounds act through the thyroid gland. In current observations activity seems to be correlated with SH groups although earlier studies demonstrated an effect of other unrelated compounds. It is also unknown whether the decreased mortality in animals fed a normal diet is due to an effect of the compounds on the liver or on other organs.

Methionine and choline. Heppel and co-workers also studied the relationship of dietary protein, fat, methionine and choline to ethylene dichloride exposure (118). A low casein or low casein-high fat diet increased mortality following single or multiple inhalations of ethylene dichloride. A supplement of choline in pair-fed rats fed a low casein, high fat diet decreased mortality after one exposure, but did not significantly change mortality following two exposures. When a lower concentration of ethylene dichloride was used, a combination of choline and methionine decreased mortality following multiple inhalations to the level obtained in control rats. Although the diet and supplements can markedly alter mortality, correlations between liver fat and mortality are less definite. Increased liver fat is not associated with increased mortality, for in single exposure studies rats may die with an average liver fat of 7.5 gm. % and survive with 12.6 gm. %. This lack of correlation is seen even more clearly following repeated exposures to ethylene dichloride, as follows:

	Av. liver fat
1. Normal diet, died	6.7 gm. %
2. Normal diet, survived	4.5 "
3. Low casein diet, died	14.8 "
4. Low casein diet, plus methionine and choline, died	7.0 "
5. Low casein diet, plus methionine and choline, survived	10.7 "

It is evident that the effect of choline and methionine in decreasing mortality is probably not related to lipotropic or other effects on the liver. As pointed out above, in animals on normal diets, ethylene dichloride produces only minimal hepatic lesions. This slight hepatotoxic effect is not enhanced by low protein or low protein-high fat diets for Heppel and associates (118) state: "In fact, the histological appearance of liver of rats dying after dichloroethane showed nothing to distinguish them from livers of rats fed similar diets but not exposed." Although it is only concluded that methionine and choline reduce mortality when added as supplements to low choline diets, statements in the literature often erroneously imply that the protective effects of methionine and choline are lipotropic in nature. The adrenal cortex may be important and should be further investigated. Ethylene dichloride produced necrosis and hemorrhage of the adrenal cortex in a large number of animals fed low casein, high fat diets but only in a few of the animals receiving the dietary supplements.

In a further study the addition of methionine, *l*-cystine or choline plus cystine to a low protein diet decreased mortality from ethylene dichloride, whereas choline, arginine, valine or alanine were without effect (121). Choline decreased liver lipids but did not affect mortality whereas cystine protected, but did not decrease the concentration of hepatic lipids. The data would seem to show that the fat content of the liver is less important than the availability of sulfhydryl groups in decreasing mortality from ethylene dichloride.

In a recent histological study, the mortality and the incidence of fatty changes following a single injection of ethylene dichloride were reduced by methionine, 1,2-dimercaptopropanol (BAL) or cysteine (124). The most consistent and pronounced reduction in the incidence of fatty changes were obtained in the heart, to a lesser extent in the liver, and least of all in the kidney.

Summary. A variety of agents will decrease the mortality of animals fed a normal diet and exposed to ethylene dichloride. The mechanism of these effects is unknown. When animals are fed low protein diets the change in liver lipids is not related to mortality. Similarly the protective effects of methionine or cystine and the lack of effect of choline, when added to low protein diets, are unrelated to changes in hepatic lipid. Such protective effects of methionine or cystine may be on the kidney or other organs of the exposed animals.

D. Propylene dichloride (1,2-dichloropropane)

Propylene dichloride is one of the more toxic halogenated hydrocarbons producing injury to various organs after oral administration (268) and particularly following inhalation (123). The inhalation studies are the most extensive; necrosis occurs in the liver, kidney and adrenal cortex.

Mortality in young rats following multiple inhalations of dichloropropane was markedly increased by a low casein diet or a low casein, high fat diet (117). The addition of choline to the low protein diet did not affect mortality whereas the addition of methionine or choline plus *l*-cystine markedly increased resistance to the effects of dichloropropane. Pathological changes were found in many organs and it is not known whether the protecting substances acted on

the liver, kidney, heart or adrenal cortex to decrease mortality, although evidence does seem to point more toward a renal effect.

E. Ethyl alcohol

Many studies have been performed to determine whether alcohol exerts a direct toxic effect on the liver. In earlier studies Opie and Alford (202) were unable to influence the mortality or produce significant hepatic lesions when single doses of alcohol were administered to rats receiving various diets. In the meantime it was shown that alcohol would increase the hepatotoxic effects of carbon tetrachloride (147) and chloroform (223). Since then a number of studies have been performed with alcohol, only a few of which will be reviewed here. Lowry and co-workers (166) reported that alcohol increased the severity of cirrhosis produced by deficient diets in pair-fed rats. In 1947 Ashworth reported that ethyl alcohol could directly produce hepatic lesions in rats (11). However, the total caloric intake of his two groups was different, for the added calories provided by the ethyl alcohol were not controlled. Further, the alcohol was administered in amounts that often produced coma, leading to a marked restriction of food intake and weight loss. The problem was re-investigated by Best *et al.* (19) who concluded that alcohol does not exert a specific toxic effect on the liver. They used pair-fed alcohol and non-alcohol rats who received isocaloric amounts of glucose in place of alcohol. The diet used contained adequate vitamin supplements, allowed a good food intake and growth of the animal, but was slightly deficient in choline and methionine. Under these conditions fatty infiltration and an early fibrosis of the liver was produced in rats receiving ethyl alcohol or isocaloric equivalents of glucose. These hepatic changes could be prevented by supplements of choline, methionine or casein. It was concluded that when the diet was marginal with respect to lipotropic factors calories in the form of alcohol or glucose would induce a relative choline deficiency producing liver injury. Further studies were reported by Klatskin and co-workers in 1951 (140). They re-investigated the problem as to whether alcohol increases choline requirement by augmenting the caloric content of the diet, and secondly whether alcohol will produce fatty infiltration of the liver when the diet is adequate with respect to protein, lipotropic factors and vitamins. In general the results are in agreement with those of Best. With the diet they used neither the alcohol nor isocaloric glucose-fed controls developed significant fatty infiltration or cirrhosis of the liver. Chemical analyses for liver fat showed only a slight increase, but still within the normal range, in the groups receiving ethyl alcohol. However, this increase in liver fat was noticed only in animals sacrificed at 117 days. No significant change in liver fat was obtained in animals sacrificed at 180 days.

It seems most likely that alcohol in doses that can be sufficiently tolerated to allow a gain in weight may produce fatty livers when the diet contains a minimal amount of choline. This effect is probably chiefly due to the calories supplied by the alcohol during a fixed intake of lipotropic factors, although it is possible that under some conditions alcohol may increase the requirement for choline by another means, perhaps by blocking in some manner its action within the liver.

Clinical studies. From the various data in the literature it can at least be said that cirrhosis of the liver is more commonly found in alcoholics than in non-drinkers. Clinically the effect of alcohol is believed to be only indirect inasmuch as it supplies calories but not lipotropic factors, decreases appetite and tends to lead to an imbalanced diet. This view is supported by the more recent experimental evidence. The treatment of the liver injury by dietary means received much impetus from the work of Patek and co-workers (206). They treated such patients with a diet high in calories and protein plus supplements of crystalline B vitamins and yeast. Such a diet will supply an adequate amount of lipotropic factors but there is no good evidence in the literature for an added effect of supplements of choline, methionine or inositol. In other studies reporting an effect of such supplements, control experiments are lacking, the need for which is particularly emphasized in a recent paper by Klatskin and Yesner (141) who obtained marked histological improvement in the liver by removal of alcohol, bed rest and an average hospital diet.

F. Benzene

The effects of chronic benzene exposure vary markedly with the diet and this relationship has been carefully studied by Li, Freeman and their associates (158, 160, 161). Dogs fed an adequate stock diet or a low fat, high protein diet were quite resistant to chronic benzene poisoning, surviving the 52-week period of study with a gain in weight. A high fat, high protein diet increased susceptibility to benzene slightly. Survival period was greatly decreased and there was loss of weight in animals on diets low in protein, independent of a low or high fat content. There was no relationship between the length of survival and the liver lipids or the level of serum phosphatase. Hepatic dye clearance (Rose Bengal test) tended to change early but was not associated with changes in liver lipids. The production of leucopenia, thrombocytopenia and anemia was most marked in animals fed the low protein diets. The percentage of conjugated sulfates in the urine increased in all animals during exposure, but most markedly in those fed the low protein diets (161).

Similar results were obtained during the chronic exposure of rats to benzene. Although benzene produced a leucopenia in all groups the change was greatest in those animals fed low protein diets (158). Total liver lipids were increased in control rats fed low protein diets, being higher than in the corresponding benzene-exposed groups. Supplements of methionine increased growth and tended to reduce the incidence of leucopenia in protein deficient rats receiving benzene (160). However, as judged by the growth curve and the number of leucocytes, the benzene exposure was still toxic. The low protein diet increased liver lipids and methionine was equally effective during benzene exposure or control exposure to air in maintaining a normal liver fat content. The expected lipotropic effect of methionine is not interfered with by benzene exposure.

Summary. A low protein diet increases the susceptibility to benzene, which may be partially counteracted by methionine. Neither the increased susceptibility to benzene nor the effect of methionine seems to be related to total liver lipids.

G. Brombenzene, methyl chloride

Brombenzene. Both brombenzene and iodobenzene are excreted in the urine as the respective halogen-phenylmercapturic acid. The incorporation of brombenzene into a low protein (six per cent) casein diet will retard growth. Supplements of cystine or methionine, which cause an increased excretion of organic sulfur, will promote growth of rats receiving brombenzene, whereas taurine or sodium sulfate is ineffective (265).

Methyl chloride. The survival of rats inhaling methyl chloride is decreased by diets low in protein. Supplements of cystine and methionine increase survival time even when added to a diet containing 20 per cent casein (244).

H. Trinitrotoluene

Although exposure to trinitrotoluene (TNT) has been reported to produce liver damage in man, it has been difficult to produce hepatic lesions with this substance in laboratory animals. In earlier studies single doses of TNT produced fatty degeneration in only two, with necrosis in one, of ten rabbits (189), although some died too early to develop hepatic lesions. The chronic administration of TNT to three cats and two rabbits failed to produce any significant change in the liver (269). Similar difficulties in producing lesions were experienced by other investigators (205, 255).

Dietary factors. In a more recent study, Himsworth and Glynn (126) were able to produce fatty changes and liver necrosis during the chronic administration of TNT to rats fed a low protein, high fat diet. The lesions were centrilobular and occasionally the necrosis was acute and accompanied by intravascular clotting in the central vein. The liver injury produced is of a delayed type for the animals dying during the first ten days were free of hepatic lesions. They concluded that the injury depends on a high fat intake inasmuch as the lesions were absent in rats receiving a high protein diet or a low protein, high carbohydrate diet. Although the two diets, low protein-high carbohydrate and low protein-high fat, contain a similar amount of protein, the protein content per 100 calories of diet will be lower in the high fat diet. Thus the injury produced by TNT could be more related to the lower protein intake per day, which would be obtained with the high fat diet.

In studies on rats, using a higher oral intake of TNT, Smith and co-workers (243) were able to produce hepatic injury in 20 per cent of the animals receiving a normal protein diet. These changes were produced with a low percentage of fat in the diet. The incidence of liver injury was not decreased by increasing the casein content of the diet to 37 per cent. It is difficult to judge from this report whether TNT added to a five per cent casein diet increased the severity of the hepatic lesions that would be expected in rats given a low (five per cent) casein diet alone for 60 days. Supplements of cystine or ascorbic acid were without effect on the liver damage. However, the addition of one per cent methionine to the diet prevented fatty livers in animals fed the low casein diet, whereas rats fed 20 per cent or 37 per cent casein diets still showed fatty changes. Methionine would seem to be of greater value than the high protein diet.

Commercial TNT contains three isomers and Barger and Tutin (12) have shown that the β and γ isomers, but not the α isomer, can combine with certain amino acids. A high protein diet might therefore offer protection by allowing the TNT to combine with protein and thus to become nontoxic. However, as pointed out by Himsworth and Glynn (126), pure α TNT produces the same changes as commercial TNT so that this possibility seems unlikely.

Clinical studies. Exposure of man to TNT may produce toxic effects among which are hepatic necrosis and fibrosis (49, 149, 249). The injury is of a delayed type, as seen in rats, and histologically is similar to that produced experimentally by Himsworth and Glynn. Eddy has reported that methionine produced remarkable improvement in patients with hepatitis due to TNT (71, 74). Although the number of cases treated is not given, some are outlined in detail. Without any controls for comparison it is difficult to say that methionine had any effect, particularly since the patients were also treated with bed rest, a high protein diet, vitamin B complex and a multiple vitamin product. (Two other patients with epidemic hepatitis were treated with methionine, which is now known to be without beneficial effect, with striking improvement in 48 hours.) From a critical reading of these publications it must be concluded that no beneficial effect of methionine on hepatitis resulting from TNT exposure has been demonstrated.

I. Pyridine

Pyridine is reported to have produced serious renal and hepatic damage following prolonged administration in two of five subjects (209). Experimentally pyridine will produce renal and hepatic injury; if the liver damage is continued for a sufficient length of time, it results in cirrhosis (16).

Diet and methionine. The addition of pyridine to a diet low in protein (10 per cent casein) rapidly affects growth and produces death with liver and renal injury (15). Increasing the amount of casein to 20 per cent or the addition of small amounts of methionine increased growth but did not affect survival. Larger amounts of methionine, even when added to a 25 per cent casein diet, increased survival time.

In further studies survival was increased by supplements of methionine, cystine, cystine plus choline, but not choline alone, when added to diets containing from 10 per cent to 25 per cent casein (17). This protective effect is not due to an increase in food intake. It is stated that the increased survival was "apparently due to prevention or reduction of liver and kidney injury," although no data on this point are given. Mention is made that choline, although not affecting mortality, did modify the hepatic lesions in the pyridine-treated animals receiving the 10 or 18 per cent casein diets. Other studies confirmed the increased survival time in pyridine-exposed animals when methionine or cystine was added to a 25 per cent casein diet, whereas choline was again ineffective (43). It is difficult to interpret the histological data presented in this paper, particularly because the authors rely more on increase in liver water as reflected in liver weights than on histological study. Liver fats, for the same duration of

exposure to pyridine, were slightly decreased by methionine and choline, but as the determination was on pooled livers the significance of the difference cannot be determined.

Although the above supplements may increase survival to toxic doses of pyridine, it cannot be stated definitely at the present time that this effect is actually related to prevention of liver or renal injury or of damage to other organs.

J. Azo compounds

Azo compounds and diet. It has been shown that various azo compounds may produce liver cancer in rats. Dimethylaminoazobenzene, first reported by Kinoshita (cf. 247), is highly active in this regard. In initial studies animals were fed diets with rice as the basal food (rice-carrot diet), so as to correspond to the diet of the rice-eating countries of the Orient, where the incidence of hepatic cancer is high. With this diet dimethylaminoazobenzene will produce hepatic tumors in 80 to 100 per cent of the animals (cf. 247). The pathological changes induced have been studied in detail (78). The development of the cancer is inhibited by supplements of rice bran extract, liver or yeast (139, 247). The addition of 18 per cent casein to the rice-carrot diet has been reported to be either without effect (247) or to decrease the incidence of the tumors (105, 139).

Using a more balanced diet, Miller *et al.* (181) obtained a protective effect with casein, liver, eggs and yeast protein. They noted that best protection against the hepatic tumors was obtained when the diet was rich in both protein and the vitamin B complex, particularly riboflavin. In one report tumors were not produced when the rats were fed synthetic diets containing six or 18 per cent casein (105). However, with a higher intake of dimethylaminoazobenzene, tumors were produced but the incidence was not significantly different in diets containing five or 37 per cent casein (241). Yeast and egg protein were protective. White and Edwards (267) also obtained tumors in rats fed a six per cent casein diet.

The riboflavin intake and content of the liver are also important, and supplements of casein and riboflavin to the rice-carrot diet markedly decreases the tumor incidence (139). Biotin is also reported to exert a procarcinogenic effect (70, 152). Variation in the amount of such substances in the diet may account for some of the above reported differences, particularly with casein supplements.

Lipotropic substances. Miller *et al.* (181) were unable to prevent the tumors by supplements of xanthine, cystine, inositol or choline. Gyorgy *et al.* (105) reported that supplements of cystine with choline, but not choline or cystine alone, prevented the occurrence of the tumor in rats fed the rice-carrot diet. However, others were unable to obtain such a protective effect with cystine, cystine plus choline, or methionine (267).

K. Phosphorus

Phosphorus produces chiefly fatty changes and necrosis in the liver but may cause parenchymatous changes in the kidney, muscle and other organs. Cirrhosis of the liver can be produced by the chronic administration of phosphorus (168).

Phosphorus and dietary factors. Opie and Alford (202) noted least mortality

with a high carbohydrate diet and the intensity of necrosis was greatest when a meat diet was fed. Rettig also observed protection with a high carbohydrate diet (216). The fatty infiltration of the liver was less with such a diet and the usual increase in nitrogenous elimination following the administration of phosphorus to fasting animals was decreased or prevented by carbohydrate. A protective effect of sugar against phosphorus poisoning is also reported (237), and Laszt and Verzar (151) state that iodoacetic acid will prevent the fatty infiltration resulting from phosphorus.

The effect of choline on such liver injury was first studied by Best, MacLean and Ridout (21). They noted in rats fed a low protein diet that choline increased the rate of disappearance of fat from the liver during the recovery phase of phosphorus poisoning but did not inhibit the deposition of fat which occurred after the administration of the phosphorus. The choline influenced only the rate of removal of fat and was without effect on the other degenerative changes produced in the liver by phosphorus.

Radioactive phosphorus and diet. Severe protein depletion, induced by feeding a diet containing 1.9 per cent protein with an associated loss of 25 per cent of body weight, increased the susceptibility to total body irradiation (138). Other studies have been reported on the subacute toxicity of radioactive phosphorus with diets varying in their casein and fat content (42). In rats fed a 10 per cent casein diet the LD_{50} of radioactive phosphorus was not influenced by fatty infiltration of the liver, induced by a deficiency of choline with or without partial poisoning with CCl_4 or by supplementing the diet with choline to prevent the fatty infiltration. Thus, although severe protein depletion may increase radiosensitivity, moderate depletion is without effect. In fact, survival following P^{32} was shortest when the diet contained 25 per cent casein. Supplements of folic acid and vitamin B_{12} had no significant effect on the survival of mice injected with P^{32} . However, when succinylsulfathiazole was added to the diet to decrease bacterial synthesis of vitamins in the intestine the animals became more susceptible to P^{32} . The administration of vitamin B_{12} and folic acid to such animals returned the survival rate nearly to normal (41).

L. Arsenic

Hepatic injury can be produced by various compounds containing arsenic, such as arsenates, arsenious oxide, arsphenamine, neoarsphenamine and dichlorophenarsine (89, 90, 113, 142, 143, 257). Large single doses of organic arsenicals can produce widespread necrosis particularly about the central and middle portion of the lobule. After repeated administration of these materials a focal necrosis is generally produced particularly in peripheral portion of the lobule. Periportal fibrosis of various degrees can also be obtained.

Arsenates and diet. The chronic administration of arsenates to rabbits will produce cirrhosis. Modification of the diet by the addition of carrots or a carbohydrate diet decreased the incidence of cirrhosis (257). In a further study with rabbits it was observed that supplements of yeast, added to a normal diet, increased survival and decreased the incidence of liver injury (256). The mecha-

nism of this effect is unknown, although it is possible that the action of the yeast is chiefly to increase food intake.

Organic arsenicals and diet. Dietary studies tend to show that a high fat intake increases the susceptibility to liver injury, although with some of the diets used the results are often difficult to interpret. In initial studies, a diet of bread, oats and milk increased survival to single doses of arsphenamine as compared with a high protein diet of lean beef heart (133). However, hepatic necrosis was not common, the chief lesion produced being necrosis of kidney epithelium. Conclusions regarding the effect of high fat diet on liver injury produced by arsphenamine are not in agreement (44, 226).

More recent studies have been performed by Hawkins and associates. They noted that arsphenamine produced severe liver injury as judged by icteric index and histologic changes when a high fat diet was fed (178). Either a protein (meat) or a carbohydrate diet was beneficial, the protein diet giving slightly better protection against hepatic damage. It is of interest that the carbohydrate diet was effective even though it contained a very low amount of protein (maximum 3.5 per cent protein). Further, the high fat diet was grossly unbalanced, being devoid of protein or carbohydrate. It was also demonstrated that methionine protected against oxophenarsine-induced liver injury in protein depleted dogs (98). All dogs were fasted for one week and then placed on a diet nearly devoid of protein for various periods. Some were also subjected to plasmaphoresis. Under such dietary conditions it is difficult to transfer the beneficial effect of methionine in experimental animals to conditions that may exist in man. Such diets will themselves produce liver injury as shown by Fouts (91), with less dietary restriction.

Clinical studies. In 1916 Westrope reported that a milk diet 24 hours before the administration of arsphenamine decreased the incidence of vomiting and headache (261). Later Peters and co-workers made a detailed study of post-arsphenamine jaundice (207). The rate of recovery of patients was observed by serial estimation of serum bilirubin and it was concluded that a slight but statistically significant increase in the rate of return to normal was obtained in patients receiving cystine or methionine. Control patients required an average of 25.7 days for the serum bilirubin to fall below four mg. %, whereas methionine-treated cases showed a similar change in an average of 19.7 days. However, in patients treated with casein the serum bilirubin did not decrease to four mg. % until 32.9 days had elapsed. Although the casein may not have supplied sufficient methionine to effect recovery, it is doubtful that it would delay recovery. If we then assume the casein to be ineffective the patients may be considered as another control group, which would then show a difference between controls (25.7 and 32.9 days) of 7.2 days. With such a variation among two control groups the difference produced by methionine would not be significant. Part of the variation is probably due to the fact that the date of onset of jaundice was elicited by questioning, and was thought in most cases to be accurate within two days.

It is probable that the jaundice studied in the above report was due to hepatic

tis virus rather than arsphenamine. The time of onset of jaundice following the start of arsphenamine therapy, the symptoms, and the clinical course of the patients are typical of homologous serum jaundice. It is now known that the virus may be easily transmitted by syringes, needles or lancets that have been in contact with infected human blood (23, 37, 224). Minute quantities of blood are infective. Methionine is without effect in patients with infectious hepatitis or homologous serum hepatitis.

M. Selenium

Grain grown in soil containing selenium, or selenium added to the diet, will produce a typical disease picture in many farm animals, causing, among other changes, damage to the liver, kidney, spleen and adrenal cortex. Details concerning selenium poisoning may be found in the review of Moxon and Rhian (194). Animals fed such materials remain normal for a few weeks, following which time some will die and show at autopsy acute massive necrosis of the liver. Others survive for longer periods and develop fibrosis of the liver, evidently the result of post-necrotic scarring. In acute toxicity studies selenium produces death by effects on the central nervous system. The subacute or chronic administration of selenium produces necrosis, cirrhosis and hemorrhage of varying degrees in rats and dogs (92, 162, 217). Depending on the dosage selenium may cause slight anorexia to complete refusal of food.

Dietary factors. Diets high, normal and low in protein have been studied and it was concluded that selenium poisoning is least severe on the diet with a high protein content. The high protein diets increased growth rates and decreased mortality (157, 193, 240, 242). Different proteins vary in protective effect (99, 242). Smith (240) and Stohlman (242) express the toxicity of selenium in relation to protein, concluding that a protein-selenium ratio (per cent protein in diet to micrograms selenium per 100 grams of diet) of 1:30 or less is non-toxic while a diet with the same selenium content but with a protein-selenium ratio of 1:100 is very toxic. The ratio of protein to selenium may account in part for differences in species response to selenium, but the problem has not been sufficiently studied. The feeding of diets with a low or high content of fat did not affect mortality from selenium (193).

Seleniferous wheat, combined with a low protein, high carbohydrate diet, resulted in a marked nodular cirrhosis but produced only slight damage when a high protein, low carbohydrate diet was fed (162, 240). However, control data on the liver lesions were not given for rats fed normal wheat combined with the low protein, high carbohydrate diet. Similar results were obtained when sodium selenite was added to synthetic diets containing various amount of protein (242), but control data on the liver injury produced by the low protein diet were lacking. In other studies cirrhosis was not obtained when seleniferous wheat was fed with a low protein, high fat diet (193). Microscopically such animals showed fatty degeneration in the liver which was not unlike that obtained, as stated in a footnote, with a similar diet and normal wheat (240). Therefore, although low protein diets increase mortality and decrease growth when selenium is fed, evi-

dence is still not conclusive that the liver injury produced is any greater than what might be expected by feeding a low protein diet alone for a prolonged period.

Cystine, methionine, choline. Cystine has consistently been without effect in counteracting the growth depressing effects or lethal action of selenium in animals fed a low protein diet (157, 193, 227, 242) or a normal diet (234). The addition of methionine to a six per cent casein diet gave some protection, as evidenced by increased growth rate and slightly longer survival, but not always to the extent obtained with a high protein diet (157). Some effect of methionine was also stated to be present in pair-fed animals. However, gain in weight and longer survival do not necessarily indicate a protective effect of the diet or dietary factors on the hepatotoxic action of selenium. Pathological changes in the liver have been noted in rats which were rapidly gaining weight and appeared otherwise normal (99). Supplements of methionine and cystine added to a low protein diet did not prevent the cirrhosis caused by selenium (242). Interesting results have been obtained by Sellers *et al.* (234) in animals fed a normal diet. They found that methionine would protect against the hepatotoxic effect of selenium, but only if alpha-tocopherol was also supplied. Supplements of choline were without effect.

It has been postulated that selenium is in part detoxified and eliminated as the volatile dimethyl selenide (132) and it is possible that methionine may serve as a source of methyl groups in this detoxification. However, in further studies it was shown that 16 to 52 per cent of injected selenium is excreted in eight hours as a volatile compound which is still unknown, the excretion of which was not influenced by choline or methionine (229).

It is known that selenium can inhibit certain cellular respiratory enzymes, but no correlation of these effects to diet has been established (cf. 240). The possibility remains that selenium produces hepatic necrosis because it replaces sulfur in sulfur-containing amino acids. In growing plants selenium can replace sulfur, for the metabolism of sulfur and selenium is similar in cereal plants, although not identical in all respects (204). The deposition of selenium in cereal proteins follows that of sulfur in most cases, but there are a few wide variations in the S/Se ratios. Selenate, however, does not seem to be formed even though a seleniferous plant is high in sulfate.

VI. POSSIBLE MECHANISMS OF DIETARY EFFECTS

Various theories have been suggested to explain the mechanism by which hepatotoxic agents produce injury, and to account for the manner by which certain diets influence the hepatic damage. Graham postulated in 1915 that the toxic effects of chloroform were due to hydrochloric acid liberated during its decomposition (101, 102). However, further investigation failed to confirm some of his results or his suggestions and the theory was soon discarded (53, 253). Other explanations have received more attention and warrant discussion in greater detail.

A. Carbohydrate and liver glycogen

Many hepatotoxic agents have been noted to decrease liver glycogen. This effect was observed as early as 1882 by Rosenbaum during experimental arsenical and phosphorus poisoning (219), and has since been reported by various investigators. However, in such studies food intake was not controlled and the fall in liver glycogen may have been due wholly or in part to a decreased food consumption following CHCl_3 anesthesia or to the administration of other toxic materials. Although the experiments were not properly controlled, such reports of low liver glycogen were used as a basis for the administration of glucose in attempts to decrease hepatotoxic reactions, particularly in man.

The studies of Rosenfeld also suggested the use of glucose to prevent liver injury in man during CHCl_3 anesthesia. He noted various conditions, including CHCl_3 poisoning, in which fatty changes in the liver were associated with a decrease in glycogen content. He believed that fat could not be metabolized unless carbohydrate was simultaneously oxidized, and therefore as liver glycogen decreased lipid would accumulate in the liver (220-222). It was concluded that a toxic substance such as CHCl_3 increased the need of the liver for carbohydrate which, when supplied, would increase the detoxifying power of the liver and prevent fatty infiltration. These data also led to the use of dextrose in patients with hepatic disease. Since then various investigators have reported that depletion of liver glycogen increases the toxicity of CHCl_3 , CCl_4 and phosphorus (25, 50, 100, 225). During this time glucose and sucrose were observed to decrease the liver injury resulting from CHCl_3 (51, 52, 100) and CCl_4 (39, 56). However, it was noted that glucose and insulin did not increase liver glycogen during phosphorus and CHCl_3 poisoning (5, 6).

The viewpoint developed by Rosenfeld went unchallenged until it was more closely studied by Ravdin and associates. It had been assumed that the decrease in liver glycogen was due to an increased rate of metabolism in the liver, but careful studies in dogs did not confirm this belief (214). They demonstrated that the administration of glucose resulted in less deposition of liver glycogen in dogs following CHCl_3 exposure than in the normal dog. Further, the protective effect of oxygen on the liver during CHCl_3 anesthesia was not associated with any effect on the rate or degree of discharge of hepatic glycogen. They believed that a decrease in liver glycogen is best explained by an inability of the damaged cells to retain glycogen, rather than by any increased metabolic activity of the liver cells. They also noted that fat began to accumulate in the liver, following CHCl_3 anesthesia, at a time when considerable hepatic glycogen was still present. Therefore, the early accumulation of fat does not seem to be solely related to a deficiency of carbohydrate, as claimed by Rosenfeld.

A further study of the relation of liver glycogen to lipid content, made by Goldschmidt, Vars and Ravdin (97), failed to confirm the hypothesis that a high content of glycogen protects the liver from CHCl_3 injury. An increased susceptibility to CHCl_3 was, however, correlated with an increase in liver fat. When rats with equal concentrations of hepatic lipids were compared the incidence of liver damage was not significantly different between groups with high

and low liver glycogen. If the content of liver fat is not considered, it may be erroneously concluded that the increase in liver injury was related to a decrease in liver glycogen. Thus, the early reports correlating a decrease in liver glycogen with increased susceptibility to hepatotoxins must be regarded as based on fortuitous results, it being more likely that the increase in toxicity was related to changes in hepatic lipids.

It is well known that the starved animal is more susceptible to liver injury (52, 239). Even when rats with the same content of hepatic lipids are compared, the animals not fed for 24 hours suffer greater liver damage than fed animals (97). From the above discussion of liver glycogen it does not seem that a deficiency of this substance is responsible for increased damage to liver when food is withheld. It is more likely that the sensitivity of the unfed animal is due to a depletion of body stores of protein, which diminish quite rapidly, particularly in the liver, of the starved rat (3, 4). CHCl_3 anesthesia will increase the excretion of nitrogenous products and this may be partially counteracted by the administration of glucose (47, 51), thus allowing a more rapid regeneration of liver cells. In fact, Opie and Alford (202, 203) initially suggested that the necrosis produced by CHCl_3 , phosphorus or other agents may be a histological expression of protein breakdown, and that carbohydrate may limit necrosis by virtue of its protein sparing action.

B. Fat storage of toxic agents

An increase in hepatic lipids, produced by dietary means, renders animals more susceptible to hepatic damage. This has been shown for a number of compounds, as discussed individually above, and is perhaps best demonstrated in the studies of Goldschmidt *et al.* (97) with CHCl_3 .

Such an effect of fatty livers, in increasing toxicity, was first noticed during the early clinical use of CHCl_3 . This led Wells to suggest in 1908 that such susceptibility to CHCl_3 may be based on the known solubility of the chemical in fats (260). He believed that a fatty liver would absorb more CHCl_3 from the blood than a normal liver and thus enable the CHCl_3 to be in contact with hepatic cells for a longer period of time and to exert a more toxic effect. Opie and Alford (202, 203) accepted this explanation and pointed out that the induced necrosis was in the region where fat was generally deposited. The studies of Goldschmidt and associates led to the same conclusion and they believed that the fat within the cells of the liver acted as a reservoir for CHCl_3 , maintaining a toxic concentration for a prolonged period of time.

Although such a hypothesis is plausible, at least for the fat-soluble materials, there are no data in the literature relative to the concentration of CHCl_3 in the liver at different levels of fat concentration. More recently McCollister *et al.* (171) studied the distribution of radioactive CCl_4 in monkeys and noted a very high concentration of radioactivity in fat. A distribution ratio was calculated and, with the ratio for blood equal to 1.00, that found for fat was 7.94, for liver 3.03, and for bone marrow 3.00. All other tissues studied had a distribution ratio of less than one. These data show the affinity of CCl_4 for fat and it would be of

interest to have similar data for the concentration of CCl_4 and related materials in the liver at different levels of hepatic lipids.

The solubility of CHCl_3 and CCl_4 in fat probably explains their increased toxicity in animals fed a high fat diet. Comparative studies are not available for other compounds, but one would suspect that solubility in fat, like the Meyer-Overton theory in anesthesia, would not hold for all compounds. In such cases where solubility in fat is a factor, the damaging agent would then be able to exert a greater toxic effect, as might be evidenced by more severe central ischemia, or by a greater effect on other cell constituents such as SH groups.

C. SH groups and dietary effects

The function of certain enzymes is known to be associated with the presence of SH groups, and it is possible that certain chlorinated hydrocarbons may exert their toxic effects by affecting such enzyme systems. The SH groups can readily be inactivated chemically, thus preventing the SH radical from functioning in an oxidation-reduction system (180, 212). It has also been reported that CHCl_3 administered orally will decrease the glutathione content of the liver (24). It is thus possible that chlorinated hydrocarbons may produce liver injury, particularly necrosis, by virtue of their ability to inactivate sulfhydryl groups.

As previously pointed out, hepatic necrosis can also be produced by a deficiency of cystine or tocopherol and it would be logical to assume that the underlying mechanism of the damage caused by these deficiencies and by toxic agents is similar. Preliminary data correlating such seemingly unrelated substances are available. Cysteine has been shown to increase the glutathione content of the liver (165). A deficiency of cystine will markedly decrease the glutathione content of the liver, which can, however, be increased by the administration of glutathione (155). Little is known about the effect of vitamin E deficiency on the SH radical although it is believed that the primary effect of alpha-tocopherol is to act as a strong reducing agent needed to preserve the reduced form of certain intracellular enzymes (122). Methionine will increase more than three-fold the antioxidant activity of alpha-tocopherol (40). Although further studies are needed, it is evident that hepatic necrosis may have a common basis related to a reduction of sulfhydryl groups caused by various hepatotoxic agents, vitamin E deficiency or cystine deficiency.

VII. DISCUSSION AND SUMMARY

A number of hepatotoxic agents have been discussed that can produce, depending on dosage and duration of administration, fatty changes, necrosis and fibrosis of the liver. Various studies, since 1925, have shown that the compounds that produce injury to the center of the liver lobule do so because a centrilobular ischemia is induced. Anoxia seems to be the most important factor in the induced ischemia, and probably acts to produce necrosis by upsetting oxidation-reduction potentials within the liver cell. The cirrhosis caused by the prolonged administration of certain compounds has also been carefully studied, with chief

emphasis being placed on the reversibility of the process during early stages and on the architectural relationship of the new connective tissue.

Dietary changes will also affect the toxicity of such agents. Diets high in fat will increase the toxicity of most of the chemicals discussed. Liver injury may also be increased under such conditions and this effect is probably due to the greater solubility of many of these substances in fat, which then allows a more prolonged toxic action of the compound.

Whereas diets high in fat increase toxicity to many drugs, best protection is obtained with diets high in carbohydrate and protein and low in fat. *However, it should not be assumed that an increase or decrease in toxicity of the hepatotoxic agents with various diets is due to an increase or decrease in the severity of liver injury.* The mortality may be affected because of changes induced in other organs such as the kidney or the heart; the adrenal gland, although little studied, seems to be affected during ethylene dichloride exposure.

Diets low in protein will produce liver injury and can also affect the toxicity of the compounds discussed, but again one should not assume that increased toxicity is synonymous with increased hepatic damage. Diets with a zero, or nearly zero content of protein can markedly increase susceptibility to substances such as CHCl_3 . Such diets, in addition to producing liver injury *per se*, can also affect other organs. It is also known that prolonged protein depletion can impair antibody production, decrease the leucocytic response of bone marrow and decrease the activity of various enzymes. Diets only moderately deficient in protein (containing about eight per cent casein), even when fed for long periods of time, either have no influence on the hepatotoxic effect of the chemical or only slightly increase the degree of liver injury. In some cases the increased injury is found in the kidney or the heart.

Depending on the hepatotoxic agent being used, its dose and route of administration, and the dietary conditions of the study, mortality may be influenced by choline, methionine, cystine, other compounds supplying SH groups, or totally unrelated agents. Changes in mortality obtained by such supplements may be independent of effects on the liver. One would expect supplements of choline to act chiefly with regard to the prevention or alleviation of fatty changes, and methionine and cystine to affect the incidence of necrosis. The effects of methionine and choline will depend on several factors. Methionine functions not only as a lipotropic agent by virtue of its labile methyl group, but also in maintenance of growth and as a source of sulfur for the formation of cystine and other sulfur-containing metabolites. These last two effects are apparently more essential to the body and sufficient methionine must be present for them before a lipotropic effect is obtained. Thus differences in response may occur depending on whether a growing rat or an adult dog is being studied. Choline can only replace the lipotropic effect of methionine. In a similar manner methionine and cystine are interchangeable maximally when the deficiency is one of organic sulfur, provided the minimal requirements for methionine are supplied and an adequate amount of choline is present. Other supplements, such as alpha-tocopherol, require further study.

The more unbalanced the diet, particularly with regard to protein deficiency, the more likely it is that various supplements will decrease the toxic effects of hepatotoxic agents, either by affecting the liver, or the kidney or other organs. In applying such results to conditions that may be present in man the experimental diet should be carefully considered. It is, for instance, unlikely that man would subsist on a protein-free diet for a period of weeks, although his diet may be moderately deficient in protein for a number of years. The various supplements may be of value in the treatment of a patient with moderate protein deficiency who has been exposed to a hepatotoxic agent, although at the present time there are no adequate clinical data on this point. With certain compounds survival has been increased by some supplements even though a normal diet was being fed to the experimental animal, and it is possible that the same effect may be obtained in man.

High carbohydrate diets or glucose injections also seem to offer protection in certain cases. The protective effect of carbohydrate is not related to maintenance of liver glycogen, but rather to a decrease in the degree of protein breakdown as measured by nitrogen excretion.

The mechanism by which the various compounds and deficient diets produce liver damage is unknown. Preliminary data tend to point to an effect on SH groups and oxidation-reduction potentials.

REFERENCES

1. ABELL, M. R., AND BEVERIDGE, J. M. R.: The production of acute massive hepatic necrosis in rats by dietary means. *Canad. J. Research*, (Sect. E.), **27**: 316-319, 1949.
2. ABELL, M. R., BEVERIDGE, J. M. R., AND FISHER, J. H.: Hepatic necrosis induced by dietary means. I. Structural changes occurring in the liver during the development of necrosis. *Arch. Path.*, **50**: 1-22, 1950.
3. ADDIS, T., POO, L. J., AND LEW, W.: The quantities of protein lost by the various organs and tissues of the body during a fast. *J. Biol. Chem.*, **115**: 111-116, 1936.
4. ADDIS, T., POO, L. J., AND LEW, W.: Protein loss from liver during a two day fast. *J. Biol. Chem.*, **115**: 117-118, 1936.
5. ALTHAUSEN, T. L.: Effects of the administration of glucose and insulin on the glycogen content of normal and experimentally damaged livers. *Ann. Int. Med.*, **6**: 193-200, 1932.
6. ALTHAUSEN, T. L., AND THOENES, E.: Influence on carbohydrate metabolism of experimentally induced hepatic changes. *Arch. Int. Med.*, **50**: 58-75, 1932.
7. ANDERSON, R. M.: Experimental pathology of the liver. II. Effect of chloroform on the normal liver and on the restored liver following partial removal. *Arch. Path.*, **14**: 335-344, 1932.
8. ANDREWS, W. H. H.: The liver lesions in malaria. *Tr. Roy. Soc. Trop. Med. & Hyg.*, **41**: 699-704, 1948.
9. ASHBURN, L. L., BAKER, W. H., AND FAULKNER, R. R.: Failure of thyroidectomy and thiouracil to protect rat liver from acute carbon tetrachloride injury. *Proc. Soc. Exper. Biol. & Med.*, **70**: 624-626, 1949.
10. ASHBURN, L. L., ENDICOTT, K. M., DAFT, F. S., AND LILLIE, R. D.: The nonportal distribution of the trabeculae in dietary cirrhosis of rats and in carbon tetrachloride cirrhosis of rats and guinea pigs. *Am. J. Path.*, **23**: 159-165, 1947.
11. ASHWORTH, C. T.: Production of fatty infiltration of liver in rats by alcohol in spite of adequate diet. *Proc. Soc. Exper. Biol. & Med.*, **66**: 382-385, 1947.
12. BARGER, G., AND TUTIN, F.: Carnosine, constitution and synthesis. *Biochem. J.*, **12**: 462-467, 1918.
13. BARRETT, H. M., BEST, C. H., MACLEAN, D. L., AND RIDOUT, J. H.: The effect of choline on the fatty liver of carbon tetrachloride poisoning. *J. Physiol.*, **97**: 103-106, 1939.
14. BARRETT, H. M., MACLEAN, D. L., AND MCHENRY, E. W.: Experiments with "antinecrotic" material prepared from liver. *J. Pharmacol. & Exper. Therap.*, **64**: 131-137, 1938.
15. BAXTER, J. H.: Studies of the mechanisms of liver and kidney injury. III. Methionine protects against damage produced in rat by diets containing pyridine. *J. Pharmacol. & Exper. Therap.*, **91**: 345-349, 1947.
16. BAXTER, J. H.: Hepatic and renal injury with calcium deposits and cirrhosis produced in rats by pyridine. *Am. J. Path.*, **24**: 503-515, 1948.
17. BAXTER, J. H.: Pyridine liver and kidney injury in rats; the influence of diet, with particular attention to methionine, cystine, and choline. *Bull. Johns Hopkins Hosp.*, **85**: 138-167, 1949.
18. BEATTIE, J., HERRERT, P. H., WECHTEL, C., AND STEELE, C. W.: Studies on hepatic dysfunction. I. Carbon tetrachloride poisoning treated with casein digest and methionine. *Brit. M. J.*, **1**: 209-211, 1944.

19. BEST, C. H., HARCROFT, W. S., LUCAS, C. C., AND RIDOUT, J. H.: Liver damage produced by feeding alcohol or sugar and its prevention by choline. *Brit. M. J.*, **2**: 1001-1006, 1949.
20. BEST, C. H., AND LUCAS, C. C.: Choline, chemistry and significance as a dietary factor. *Vitamins and Hormones*, **1**: 1-58, 1943. Academic Press, Inc., New York.
21. BEST, C. H., MACLEAN, D. L., AND RIDOUT, J. H.: Choline and liver fat in phosphorus poisoning. *J. Physiol.*, **83**: 275-284, 1935.
22. BEVAN, A. D., AND FAVILL, H. B.: Acid intoxication and late poisonous effects of anesthetics. Hepatic toxemia. Acute fatty degeneration of the liver following chloroform and ether anesthesia. *J. Am. M. A.*, **45**: 691-696, 754-759, 1905.
23. BIGGER, J. W.: Jaundice in syphilis under treatment: possible transmission of virus. *Lancet*, **1**: 457-458, 1943.
24. BINET, L., WELLER, G., AND GOUDARD, H.: Le taux du glutathion dans le foie altéré. *Compt. rend. Soc. biol.*, **126**: 1141-1143, 1937.
25. BOLLMAN, J. L.: Experimental observations on glucose as a therapeutic agent. *Surg. Clin. North America*, **5**: 871-879, 1925.
26. BOLLMAN, J. L.: Some experimental observations pertinent to the treatment of hepatic disease. *Ann. Int. Med.*, **12**: 1-5, 1938.
27. BOLLMAN, J. L.: The protective value of foods in experimental cirrhosis. *Proc. Central Soc. Clin. Research*, **15**: 26-27, 1942.
28. BOLLMAN, J. L.: Protective value of foods in experimental cirrhosis. *J. Am. M. A.*, **121**: 1413-1413, 1943.
29. BOLLMAN, J. L., AND MANN, F. C.: Experimentally produced lesions of the liver. *Ann. Int. Med.*, **5**: 699-711, 1931.
30. BRUNNENBERG, A., JOHNSON, C., AND NICHOLS, S.: Carbon tetrachloride injury of the liver. The protective action of certain compounds. *Proc. Soc. Exper. Biol. & Med.*, **60**: 388-391, 1945.
31. BRUNNENBERG, A., NICHOLS, S., BIGELOW, R. R., AND MILES, J.: Sulfhydryl protection of the liver. *Arch. Path.*, **40**: 81-83, 1945.
32. CALDER, R. M.: The influence of vitamin B deficiency on experimental liver necrosis. *J. Path. & Bact.*, **54**: 355-368, 1942.
33. CAMERON, G. R., AND KARUNARATNE, W. A. E.: Carbon tetrachloride cirrhosis in relation to liver regeneration. *J. Path. & Bact.*, **42**: 1-21, 1936.
34. CAMERON, G. R., KARUNARATNE, W. A. E., AND THOMAS, J. C.: Massive necrosis ("Toxic infarctions") of the liver following intra-portal administration of poisons. *J. Path. & Bact.*, **44**: 297-303, 1937.
35. CAMERON, G. R., AND MAYES, B. T.: Ligation of the hepatic artery. *J. Path. & Bact.*, **33**: 799-831, 1930.
36. CAMPBELL, R. M., AND KOSTERLITZ, H. W.: The effects of short-term changes in dietary protein on the response of the liver to carbon tetrachloride injury. *Brit. J. Exper. Path.*, **29**: 149-159, 1948.
37. CAPPE, R. B., SBOBOV, V., AND SCHEFFLEY, C. S.: Syringe-transmitted epidemic of infectious hepatitis: with observations regarding incidence and nature of infected donors. *J. Am. M. A.*, **136**: 819-824, 1948.
38. CASPER, J. L.: Drei neue Fälle von Chloroform-Tod. *Wehnschr. f. d. ges. Heilk.*, **16**: 273-281, 1890.
39. CHANDLER, A. C., AND CHOPRA, R. N.: Effects of the administration of sugar, magnesium sulfate, sodium citrate and dilute acid on the liver damage done by carbon tetrachloride. *Indian J. M. Research*, **14**: 219-226, 1926.
40. CLAUSSEN, F., LUNDBERG, W. O., AND BURR, G. O.: Some effects of amino acids and certain other substances on lard containing phenolic antioxidants. *J. Am. Oil Chemist's Soc.*, **24**: 403-404, 1947.
41. CORNATZER, W. E., ARTOM, C., HARRELL, G. T., AND CAYER, D.: Beneficial effects of vitamin B₁₂ and folic acid on recovery from internal radiation by P³². *Proc. Soc. Exper. Biol. & Med.*, **76**: 552-554, 1951.
42. CORNATZER, W. E., HARRELL, G. T., JR., CAYER, D., AND ARTOM, C.: Subacute toxicity of radioactive phosphorus as related to the composition of diet. *Proc. Soc. Exper. Biol. & Med.*, **73**: 492-495, 1950.
43. COULSON, R. A., AND BRAIDA, F. G.: The influence of choline, cystine, and methionine on toxic effects of pyridine and certain related compounds. *Proc. Soc. Exper. Biol. & Med.*, **69**: 480-487, 1948.
44. CRAVEN, E. B.: The importance of diet in preventing acute yellow atrophy during arsenamine treatment. *Bull. Johns Hopkins Hosp.*, **48**: 131-142, 1931.
45. CUTLER, J. T.: The accumulation of guanidine in the blood following acute liver injury by carbon tetrachloride, chloroform, arsenic or phosphorus. *J. Pharmacol. & Exper. Therap.*, **41**: 337-345, 1931.
46. CUTLER, J. T.: The influence of diet on carbon tetrachloride intoxication in dogs. *J. Pharmacol. & Exper. Therap.*, **45**: 209-226, 1932.
47. DAFT, F. S., ROSSCHIT-ROBBINS, F. S., AND WHIPPLE, G. H.: Liver injury by chloroform, nitrogen metabolism, and conservation. Liver function and hemoglobin production in anemia. *J. Biol. Chem.*, **113**: 391-404, 1936.
48. DAFT, F. S., SERRELL, W. H., AND LILLIE, R. D.: Prevention by cystine or methionine of hemorrhage and necrosis of the liver in rats. *Proc. Soc. Exper. Biol. & Med.*, **50**: 1-5, 1942.
49. DAVIE, T. B.: Discussion on trinitrotoluene poisoning. *Proc. Roy. Soc. Med.*, **35**: 558-559, 1942.
50. DAVIE, N. C.: The influence of diet upon the liver injury produced by carbon tetrachloride. *J. Med. Research*, **44**: 601-614, 1924.
51. DAVIE, N. C., HALL, C. C., AND WHIPPLE, G. H.: The rapid construction of liver cell protein on a strict carbohydrate diet contrasted with fasting. *Arch. Int. Med.*, **23**: 689-710, 1919.
52. DAVIE, N. C., AND WHIPPLE, G. H.: The influence of fasting and various diets on the liver injury effected by chloroform anesthesia. *Arch. Int. Med.*, **23**: 612-635, 1919.
53. DAVIS, N. C., AND WHIPPLE, G. H.: The influence of drugs and chemical agents on the liver necrosis of chloroform anesthesia. *Arch. Int. Med.*, **23**: 636-654, 1919.
54. DAVIS, N. C., AND WHIPPLE, G. H.: Liver regeneration following chloroform injury as influenced by various diets. *Arch. Int. Med.*, **23**: 711-722, 1919.

55. DENT, C. E.: The cystine and methionine contents of liver protein in acute hepatic necrosis. *Biochem. J.*, **41**: 319-320, 1947.
56. DILLARD, G. H. L., SPENCE, H. Y., and FORBES, J. C.: Effect of food on liver fat of animals following carbon tetrachloride poisoning. *Virginia M. Monthly*, **71**: 154-158, 1944.
57. DRILL, V. A.: Changes in liver function during experimentally induced human hepatitis. *Yale J. Biol. & Med.*, **18**: 345-358, 1946.
58. DRILL, V. A.: Effect of moderate protein depletion on liver injury produced by CHCl_3 in the dog. (To be published.)
59. DRILL, V. A., and BONNYCASTLE, D. D.: Relation of methionine and protein intake to chloroform induced injury in the rat. (To be published.)
60. DRILL, V. A., and HALL, C. A.: Lipotropic effect of liver extract and other supplements in dietary induced liver injury in rats. *Am. J. M. Sc.*, **219**: 197-205, 1950.
61. DRILL, V. A., and HAYS, H. W.: Studies on the relation of the liver function, pulse rate and temperature of hyperthyroid dogs to vitamin B_3 and yeast. *Am. J. Physiol.*, **136**: 762-771, 1942.
62. DRILL, V. A., and IVY, A. C.: Comparative value of bromsulphalein, serum phosphatase, prothrombin time, and intravenous galactose tolerance tests in detecting hepatic damage produced by carbon tetrachloride. *J. Clin. Investigation*, **23**: 209-216, 1944.
63. DRILL, V. A., and LAIRD, R. D.: Variation in lipotropic activity of vitamin B_3 concentrates. (To be published.)
64. DRILL, V. A., and LOOMIS, T. A.: Effect of methionine supplements on hepatic injury produced by carbon tetrachloride. *Science*, **103**: 199-201, 1946.
65. DRILL, V. A., and LOOMIS, T. A.: Methionine therapy in experimental liver injury produced by carbon tetrachloride. *J. Pharmacol. & Exper. Therap.*, **90**: 138-149, 1947.
66. DRILL, V. A., and LOOMIS, T. A.: Production of functional and fatty changes in the liver by a chronic vitamin B complex deficiency, and interrelationships with protein intake. *J. Nutrition*, **35**: 333-350, 1948.
67. DRILL, V. A., LOOMIS, T. A., and BELFORD, J.: Effect of protein and carbohydrate intake on liver injury produced in dogs by carbon tetrachloride. *J. Indust. Hyg. & Toxicol.*, **29**: 180-184, 1947.
68. DRILL, V. A., and MCCORMICK, H. M.: Lipotropic effects of vitamin B_3 concentrate. *Proc. Soc. Exper. Biol. & Med.*, **72**: 388-390, 1949.
69. DRILL, V. A., and SHAFFER, C. B.: Serum cholesterol, serum phosphatase, and bromsulphalein retention in experimental hyperthyroidism. *Gastroenterology*, **1**: 308-315, 1943.
70. DU VIGNEAUD, V., SPANGLER, J. M., BURK, D., KESSLER, C. J., SUGIURA, K., and RHODAS, C. P.: The procarcinogenic effect of biotin in butter yellow tumor formation. *Science*, **95**: 174-176, 1942.
71. EDDY, J. H., JR.: Methionine in the treatment of toxic hepatitis. *Am. J. M. Sc.*, **216**: 374-380, 1945.
72. EDDY, J. H., JR.: Carbon tetrachloride poisoning in industry. *Indust. Med.*, **14**: 283-285, 1945.
73. EDDY, J. H., JR.: Carbon tetrachloride poisoning; a preliminary report on the use of methionine in hepatitis. *J. Am. M. A.*, **128**: 994-996, 1945.
74. EDDY, J. H., JR.: Nutritional factors in prevention and treatment of the hepatic injury due to industrial poisons. *South. M. J.*, **40**: 171-175, 1947.
75. EDWARDS, J. E.: Hepatomas in mice induced with carbon tetrachloride. *J. Nat. Cancer Inst.*, **2**: 197-199, 1941.
76. EDWARDS, J. E., and DALTON, A. J.: Induction of cirrhosis of the liver and of hepatomas in mice with carbon tetrachloride. *J. Nat. Cancer Inst.*, **3**: 19-41, 1942.
77. EDWARDS, J. E., HESTON, W. E., and DALTON, A. J.: Induction of the carbon tetrachloride hepatoma in strain L mice. *J. Nat. Cancer Inst.*, **23**: 297-301, 1942.
78. EDWARDS, J. E., and WHITE, J.: Pathologic changes, with special reference to pigmentation and classification of hepatic tumors in rats fed p-Dimethylaminoazo benzene. *J. Nat. Cancer Inst.*, **2**: 157-183, 1942.
79. ESCHENROEDER, A. B., and MILLER, E.: Liver necrosis and the induction of carbon tetrachloride hepatomas in strain A mice. *J. Nat. Cancer Inst.*, **6**: 325-341, 1946.
80. FAZKAS, J. F., ALEXANDER, A. D., and HIMWICH, H. E.: Tolerance of the newborn to anoxia. *Am. J. Physiol.*, **134**: 281-287, 1941.
81. FORBES, H. W.: Cirrhosis of the liver in rabbits with continued chloroform poisoning and with associated syphilitic infection. *Arch. Path.*, **26**: 1023-1028, 1938.
82. FITZHUGH, O. G.: Liver regeneration in rats protected with xanthine against carbon tetrachloride poisoning. *Proc. Soc. Exper. Biol. & Med.*, **40**: 11-15, 1939.
83. FORBES, J. C.: Studies on prevention of liver cirrhosis by the subcutaneous injections of xanthine containing preparations. *J. Pharmacol. & Exper. Therap.*, **65**: 287-293, 1939.
84. FORBES, J. C., and EVANS, E. I.: Protective action of sulfanilamide against hepatic damage from chloroform inhalation. *War Med.*, **4**: 418-421, 1943.
85. FORBES, J. C., LEACH, B. E., and outhouse, E. L.: Studies on fat metabolism and susceptibility to carbon tetrachloride. *J. Pharmacol. & Exper. Therap.*, **72**: 202-210, 1941.
86. FORBES, J. C., LEACH, B. E., and WILLIAMS, G.: Protective action of sulfanilamide against liver cirrhosis from chronic poisoning with carbon tetrachloride. *Proc. Soc. Exper. Biol. & Med.*, **51**: 47-48, 1942.
87. FORBES, J. C., and McCONNELL, J. S.: Crystallization of liver fraction protecting against necrosis from carbon tetrachloride or chloroform administration. *Proc. Soc. Exper. Biol. & Med.*, **36**: 359-360, 1937.
88. FORBES, J. C., NEALE, R. C., and SCHERER, J. H.: Liver preparation protecting against necrosis from chloroform or carbon tetrachloride administration. *J. Pharmacol. & Exper. Therap.*, **58**: 402-408, 1936.
89. FOULERTON, A. G. R.: On poisoning by arsenobenzol compounds used in the treatment of syphilis. *Brit. M. J.*, **1**: 864-867, 1920.

90. FOULERTON, A. G. R.: On acute yellow atrophy of the liver and the fatty infiltration of the liver and kidney which results from the action of certain poisons on the liver. *J. Path. & Bact.*, **24**: 257-271, 1921.
91. FOUTS, P. J.: Vitamin B complex studies in dogs: Production of cirrhosis of liver. *J. Nutrition*, **25**: 217-228, 1943.
92. FRANK, K. W., AND PORTER, V. R.: The effect of selenium containing foodstuffs on growth and re- reduction of rats at various ages. *J. Nutrition*, **12**: 205-214, 1936.
93. GARDNER, G. H., GROBE, R. C., GUSTAFSON, R. K., MAINE, E. D., THOMPSON, M. J., WELLS, H. S., AND LAMSON, P. D.: Studies on the pathological histology of experimental carbon tetrachloride poisoning. *Bull. Johns Hopkins Hosp.*, **36**: 107-133, 1925.
94. GLYNN, L. E., AND HIMSWORTH, H. P.: The intralobular circulation in acute liver injury. *Clin. Sci.*, **6**: 235-241, 1948.
95. GLYNN, L. E., HIMSWORTH, H. P., AND NEUBERGER, A.: Pathological states due to deficiency of the sulphur containing amino acids. *Brit. J. Exper. Path.*, **26**: 325-337, 1945.
96. GOLDSCHMIDT, S., RAYDIN, I. S., AND LUCKE, B.: Anesthesia and liver damage. I. The protective action of oxygen against the necrotizing effect of certain anesthetics on the liver. *J. Pharmacol. & Exper. Therap.*, **59**: 1-14, 1937.
97. GOLDSCHMIDT, S., VARS, H. M., AND RAYDIN, I. S.: The influence of the foodstuffs upon the susceptibility of the liver to injury by chloroform, and the probable mechanisms of their action. *J. Clin. Investigation*, **18**: 277-289, 1939.
98. GOODSELL, J. P. B., HANSON, P. C., AND HAWKINS, W. B.: Methionine protects against mapharsen liver injury in protein-depleted dogs. *J. Exper. Med.*, **79**: 625-632, 1944.
99. GOETTER, R. A., JR.: Chronic selenium poisoning of rats as influenced by dietary protein. *J. Nutrition*, **19**: 105-112, 1940.
100. GRAHAM, E. A.: The resistance of pups to late chloroform poisoning in its relation to liver glycogen. *J. Exper. Med.*, **21**: 185-191, 1915.
101. GRAHAM, E. A.: Late poisoning with chloroform and other alkyl halides in relationship to the halogen acids formed by their chemical dissociation. *J. Exper. Med.*, **22**: 48-75, 1915.
102. GRAHAM, E. A.: Toxic factors of some of the common anesthetic substances. *J. Am. M. A.*, **69**: 1666-1668, 1917.
103. GRIFFITH, F. R., AND EMORY, F. E.: The vasomotor control of the liver circulation. *Am. J. Physiol.*, **95**: 29-34, 1930.
104. GYORGY, P., AND GOLDBLATT, H.: Further observations on the production and prevention of dietary hepatic injury in rats. *J. Exper. Med.*, **89**: 245-268, 1949.
105. GYORGY, P., POLING, E. C., AND GOLDBLATT, H.: Necrosis, cirrhosis and cancer of liver in rats fed a diet containing dimethylaminobenzene. *Proc. Soc. Exper. Biol. & Med.*, **47**: 41-44, 1941.
106. GYORGY, P., AND ROSE, C. S.: Effect of vitamin B₁₂ on experimental hepatic injury. *Proc. Soc. Exper. Biol. & Med.*, **73**: 372-375, 1950.
107. GYORGY, P., ROSE, C. S., AND GOLDBLATT, H.: Prevention of experimental dietary hepatic cirrhosis by goitrogenic substances. *Proc. Soc. Exper. Biol. & Med.*, **67**: 67-70, 1948.
108. GYORGY, P., SEIFTER, J., TOMARELLI, R. M., AND GOLDBLATT, H.: Influence of dietary factors and sex on the toxicity of carbon tetrachloride in rats. *J. Exper. Med.*, **83**: 449-462, 1946.
109. HALL, C. A., AND DRILL, V. A.: Lipotropic effect of liver extract on dietary hepatic injury in rats. *Proc. Soc. Exper. Biol. & Med.*, **69**: 3-6, 1948.
110. HALL, C. A., AND DRILL, V. A.: Relation of fat and protein intake to fatty changes, fibrosis and necrosis of the liver. *Proc. Soc. Exper. Biol. & Med.*, **70**: 292-297, 1949.
111. HALL, M. C.: The use of carbon tetrachloride for the removal of hookworms. *J. Am. M. A.*, **77**: 1641-1643, 1921.
112. HALL, M. C.: Carbon tetrachloride as an anthelmintic. *Am. J. Trop. Med.*, **2**: 373-377, 1922.
113. HALL, M. C., AND SHILLINGER, J. E.: Miscellaneous tests of carbon tetrachloride as an anthelmintic. *J. Agr. Res.*, **23**: 163-191, 1923.
114. HANDLER, P., AND DURIN, I. N.: The significance of fatty infiltration in the development of hepatic cirrhosis due to choline deficiency. *J. Nutrition*, **31**: 141-157, 1946.
115. HANDLER, P., AND FOLLIS, R. H.: The role of thyroid activity in the pathogenesis of hepatic lesions due to choline and cystine deficiency. *J. Nutrition*, **35**: 669-686, 1948.
116. HEFFEL, L. A.: Private communication.
117. HEFFEL, L. A., HIGHMAN, B., AND PORTERFIELD, V. T.: Toxicology of 1,2-dichloropropane (propylenedichloride). II. Influence of dietary factors on the toxicity of dichloropropane. *J. Pharmacol. & Exper. Therap.*, **87**: 11-17, 1946.
118. HEFFEL, L. A., NEAL, P. A., DAFT, F. S., ENDICOTT, K. M., ORR, M. L., AND PORTERFIELD, V. T.: Toxicology of 1,2-dichloroethane (Ethylene dichloride). II. Influence of dietary factors on the toxicity of dichloroethane. *J. Indust. Hyg. & Toxicol.*, **27**: 15-21, 1945.
119. HEFFEL, L. A., NEAL, P. A., PENNIN, T. L., ENDICOTT, K. M., AND PORTERFIELD, V. T.: The toxicology of 1,2-dichloroethane (Ethylene dichloride). III. Its acute toxicity and the effect of protective agents. *J. Pharmacol. & Exper. Therap.*, **84**: 53-63, 1945.
120. HEFFEL, L. A., PORTERFIELD, V. T., AND PRAKE, E. G.: The lipotropic activity of caffeine, theobromine and theophylline. *Arch. Biochem.*, **15**: 439-443, 1947.
121. HEFFEL, L. A., PORTERFIELD, V. T., AND SHARPLESS, N. E.: Toxicology of 1,2-dichloroethane (Ethylene dichloride). IV. Its detoxification by L-cystine, dl-methionine and certain other sulfur containing compounds. *J. Pharmacol. & Exper. Therap.*, **91**: 385-394, 1947.
122. HICKMAN, K. C. D., AND HARRIS, P. L.: Tocopherol interrelationships. *Advances in Enzymology*, **6**: 469-524, 1946.

123. HIGHMAN, B., AND HEFFEL, L. A.: Toxicology of 1,2-dichloropropane (Propylene dichloride). III. Pathologic changes produced by a short series of daily exposures. *Arch. Path.*, **42**: 525-534, 1946.
124. HIGHMAN, B., HEFFEL, L. A., AND LAMPREY, R. J.: The toxicology of 1-2 dichloroethane (ethylene dichloride). V. Effect of protective agents on visceral fatty changes in exposed rats. *Arch. Path.*, **51**: 346-350, 1951.
125. HIMS WORTH, H. P.: The liver and its diseases. Harvard University Press, Cambridge, Mass., 2nd edition, 1950.
126. HIMS WORTH, H. P., AND GLYNN, L. E.: Experimental trinitrotoluene poisoning: the effect of diet. *Clin. Sc.*, **4**: 421-443, 1942.
127. HIMS WORTH, H. P., AND GLYNN, L. E.: Massive hepatic necrosis and diffuse hepatic fibrosis (acute yellow atrophy and portal cirrhosis): Their production by means of diet. *Clin. Sc.*, **5**: 93-123, 1944.
128. HIMS WORTH, H. P., AND GLYNN, L. E.: The prevention of experimental massive hepatic necrosis by methionine. *Clin. Sc.*, **5**: 133-137, 1944.
129. HIMS WORTH, H. P., AND LINDAN, O.: Dietetic necrosis of the liver: the influence of a tocopherol. *Nature, London*, **163**: 30-30, 1949.
130. HOCK, A., AND FINK, H.: Ueber eine schwere ernährungsbedingte Stoffwechselstörung und ihre Verhütung durch Cystin. *Ztschr. f. physiol. Chem.*, **278**: 136-142, 1943.
131. HOCK, A., AND FINK, H.: Ueber den biologischen Ergänzungswert verschiedener Nahrungsproteine. IV. Ueber die Bedeutung des Cystins für den Stoffwechsel, zugleich ein Beitrag zur Verbesserung des Hefeeiweisses. *Ztschr. f. physiol. Chem.*, **279**: 187-206, 1943.
132. HOEFMEISTER, F.: Ueber Methylierung im Tierkörper. *Arch. exper. Path. u. Pharmacol.*, **33**: 198-215, 1894.
133. HOOPER, C. W., KOLLS, A. C., AND WRIGHT, K. D.: Influence of fasting and various diets on arspenamine poisoning and the comparative toxicity of arspenamine, nearsphenamine and para-oxy-meta-amino-phenylarsenoxide. *J. Pharmacol. & Exper. Therap.*, **18**: 133-153, 1921.
134. HOUTGH, V. H., AND FREEMAN, S.: The effect of a protein-deficient diet on the serum phosphatase and hepatic dye clearance of dogs. *Am. J. Physiol.*, **133**: 184-189, 1942.
135. HOUTGH, V. H., MONAHAN, E. P., LI, T. W., AND FREEMAN, S.: The effect of choline and cystine on the serum phosphatase and hepatic dye clearance of dogs maintained on deficient diets. *Am. J. Physiol.*, **139**: 642-651, 1943.
136. HOVE, E. L.: Interrelation between tocopherol and protein metabolism. III. The protective effect of vitamin E, and certain nitrogenous compounds against CCl₄ poisoning in rats. *Arch. Biochem.*, **17**: 467-474, 1948.
137. HOWLAND, J., AND RICHARDS, A. N.: An experimental study of the metabolism and pathology of delayed chloroform poisoning. *J. Exper. Med.*, **11**: 344-372, 1909.
138. JENNINGS, F. L.: Effect of protein depletion upon susceptibility of rats to total body irradiation. *Proc. Soc. Exper. Biol. & Med.*, **72**: 487-491, 1949.
139. KENSLER, C. J., SUGIURA, K., YOUNG, N. F., HALTER, C. R., AND RHOADS, C. P.: Partial protection of rats by riboflavin with casein against liver cancer caused by dimethylaminobenzene. *Science*, **93**: 308-310, 1941.
140. KLATSKIN, G., GEWIN, H. M., AND KREHL, W. A.: Effects of prolonged alcohol ingestion on the liver of the rat under conditions of controlled adequate dietary intake. *Yale J. Biol. & Med.*, **23**: 317-331, 1951.
141. KLATSKIN, G., AND VESNER, R.: Factors in the treatment of Laennec's cirrhosis: I. Clinical and histological changes observed during a period of bedrest, alcohol withdrawal and a minimal basic diet. *J. Clin. Investigation*, **28**: 723-735, 1949.
142. KOLMER, J. A., AND LUCKE, B.: A study of the histologic changes produced experimentally in rabbits by arspenamine. *Arch. Dermat. & Syph.*, **3**: 483-514, 1921.
143. KOLMER, J. A., AND LUCKE, B.: A study of the histologic changes produced experimentally in rabbits by nearsphenamine. *Arch. Dermat. & Syph.*, **3**: 515-530, 1921.
144. LACQUET, A. M.: Experimental pathology of the liver. VIII. Effects of carbon tetrachloride on the normal and restored liver after partial hepatectomy. *Arch. Path.*, **14**: 164-176, 1932.
145. LAIRD, R. D., MCCORMICK, H. M., AND DRILL, V. A.: Lipotropic activity of folic acid, citrovorum factor, choline and vitamin B₁₂. *Proc. Soc. Exper. Biol. & Med.*, (In press.)
146. LAKE, G. C.: Carbon tetrachloride. A drug proposed for the removal of hookworms, with special reference to its toxicity for monkeys when given by stomach tube in repeated doses. *Pub. Health Rep.*, **37**: 1123-1126, 1922.
147. LAMSON, P. D., GARDNER, G. H., GUSTAFSON, R. K., MAIRE, E. D., McLEAN, A. J., AND WELLS, H. S.: The pharmacology and toxicology of carbon tetrachloride. *J. Pharmacol. & Exper. Therap.*, **22**: 215-288, 1923.
148. LAMSON, P. D., AND WING, R.: Early cirrhosis of the liver produced in dogs by carbon tetrachloride. *J. Pharmacol. & Exper. Therap.*, **29**: 191-202, 1926.
149. LANE, R. E.: Discussion on trinitrotoluene poisoning. *Proc. Roy. Soc. Med.*, **35**: 556-557, 1942.
150. LANGENBECK, J., in BEREND, N.: Zur Chloroform-Casuistik, Hannover, Hahn, 1850.
151. LAZET, L., AND VERZAR, F.: Beeinflussung der Fettwanderung durch Jodessigsäure und Nebennierensexstirpation. *Biochem. Ztschr.*, **285**: 356-367, 1936.
152. LAURENCE, W. L.: Induced biotin deficiency as a possible explanation of observed spontaneous recessions in malignancy. *Science*, **94**: 88-89, 1941.
153. LAZAREW, N. W.: Ueber die narkotische Wirkungskraft der Dämpfe der Chloride derivate des Methans, des Äthans und des Äthylens. *Arch. exper. Path. u. Pharmacol.*, **141**: 19-24, 1929.
154. LEACH, B. E., AND FORBES, J. C.: Sulfonamide drugs as protective agents against carbon tetrachloride poisoning. *Proc. Soc. Exper. Biol. & Med.*, **48**: 361-363, 1941.
155. LEAF, G., AND NEUBERGER, A.: The effect of diet on the glutathione content of the liver. *Biochem. J., Proc.*, **41**: xx, 1947.
156. LEHNHERR, E. R.: Acute carbon tetrachloride poisoning. *Arch. Int. Med.*, **56**: 98-104, 1935.

157. LEWIS, H. B., SCHULTZ, J., AND GORTNER, R. A., JR.: Dietary protein and the toxicity of sodium selenite in the white rat. *J. Pharmacol. & Exper. Therap.*, **68**: 292-299, 1940.
158. LI, T. W., AND FREEMAN, S.: The effect of protein and fat content of the diet upon the toxicity of benzene for rats. *Am. J. Physiol.*, **145**: 158-165, 1945.
159. LI, T. W., AND FREEMAN, S.: Effect of protein deficiency and cholesterol feeding on the liver of dogs. *Am. J. Physiol.*, **145**: 646-659, 1946.
160. LI, T. W., AND FREEMAN, S.: The effect of methionine on protein-deficient rats exposed to benzene. *Am. J. Physiol.*, **148**: 358-364, 1947.
161. LI, T. W., FREEMAN, S., HOUGH, V. H., AND GUNN, F. D.: The increased susceptibility of protein-deficient dogs to benzene poisoning. *Am. J. Physiol.*, **145**: 166-176, 1945.
162. LILLIE, R. D., AND SMITH, M. I.: Histogenesis of hepatic cirrhosis in chronic food selenosis. *Am. J. Path.*, **16**: 223-228, 1940.
163. LOEFFLER, L.: Factors determining necrosis or survival of liver tissue after ligation of hepatic artery. *Arch. Path.*, **21**: 496-503, 1936.
164. LOEFFLER, L., AND NORDMANN, M. LEBERSTUDIEN.: I. Die Leber bei der Verdauung von Normalkost, nach Fett-, Glykogen- und Eiweissfütterung, im Hungerzustande und unter der Einwirkung von Adrenalin, Chloroform, Phosphor, Phlorizin und Insulin. Nach mikroskopischen Untersuchungen der Leber lebender Säugetiere. *Arch. f. path. anat.*, **257**: 119-181, 1925.
165. LOEFFLER, M., COTTET, J., AND ESCALLIER, G.: Variations respectives du glutathion et de l'acide ascorbique dans le foie. *Compt. rend. Soc. biol.*, **125**: 502-504, 1937.
166. LOWRY, J. V., ASHURN, L. L., DAPT, F. S., AND SEBRELL, W. H.: Effect of alcohol in experimental liver cirrhosis. *Quart. J. Stud. Alcohol*, **3**: 168-175, 1942.
167. MACNIDER, W. B.: A consideration of the acquired resistance of fixed tissue cells to injury. *Ann. Int. Med.*, **12**: 147-165, 1938.
168. MALLORY, F. B.: Phosphorus and alcoholic cirrhosis. *Am. J. Path.*, **9**: 557-567, 1933.
169. MANN, F. C., FISHBACH, F. C., GAY, J. G., AND GREEN, G. F.: Experimental pathology of the liver. *Arch. Path.*, **12**: 787-793, 1931.
170. MARTIN, G. H., BUNTING, C. H., AND LOWENHART, A. S.: The morphological changes in the tissues of the rabbit as a result of reduced oxidations. *J. Pharmacol. & Exper. Therap.*, **8**: 112-115, 1916.
171. MCCOLLISTER, D. D., BEAMER, W. H., ATCHISON, G. J., AND SPENCER, H. C.: The absorption, distribution and elimination of radioactive carbon tetrachloride by monkeys upon exposure to low vapor concentration. *J. Pharmacol. & Exper. Therap.*, **102**: 112-124, 1951.
172. MCCORMICK, H. M., AND DRILL, V. A.: Lipotropic effects of liver extract, vitamin B₁₂ and choline. *Proc. Soc. Exper. Biol. & Med.*, **74**: 626-630, 1950.
173. MCHENRY, E. W., AND PATTERSON, J. M.: Lipotropic factors. *Physiol. Rev.*, **24**: 128-167, 1944.
174. MCIVER, M. A.: Increased susceptibility to chloroform poisoning produced in the albino rat by injection of crystalline thyroxine. *Proc. Soc. Exper. Biol. & Med.*, **45**: 201-206, 1940.
175. MCIVER, M. A., AND WINTER, E. A.: Further studies on increased susceptibility to chloroform poisoning produced in the albino rat by injection of crystalline thyroxine. *J. Clin. Investigation*, **21**: 191-196, 1942.
176. MCIVER, M. A., AND WINTER, E. A.: Deleterious effects of anoxia on the liver of the hyperthyroid animal. *Arch. Surg.*, **46**: 171-185, 1943.
177. MCMICHAEL, J.: The oxygen supply of the liver. *Quart. J. Exper. Physiol.*, **27**: 73-87, 1937.
178. MESSINGER, W. J., AND HAWKINS, W. B.: Arspenamine liver injury modified by diet. Protein and carbohydrate protective, but fat injurious. *Am. J. M. Sc.*, **199**: 216-225, 1940.
179. MEYER, J. R., AND PESSOA, S. B.: A study on the toxicity of carbon tetrachloride. *Am. J. Trop. Med.*, **3**: 177-196, 1923.
180. MICHAELIS, L., AND SCHUBERT, M. P.: The reaction of iodoacetic acid on mercaptans and amines. *J. Biol. Chem.*, **106**: 331-341, 1934.
181. MILLER, J. A., MINER, M. S., RUSCH, H. P., AND BAUMANN, C. A.: Diet and hepatic tumor formation. *Cancer Research*, **1**: 699-708, 1941.
182. MILLER, L. L., ROGERS, C. A., LAMSON, B. G., AND LAMBIE, M. W.: Protein depletion and hepatic function in dog. Rationale of Methionine in therapy. *Am. J. M. Sc.*, **214**: 84-88, 1947.
183. MILLER, L. L., ROSS, J. F., AND WHIPPLE, G. H.: Methionine and cystine specific protein factors preventing chloroform liver injury in protein-depleted dogs. *Am. J. M. Sc.*, **200**: 739-756, 1940.
184. MILLER, L. L., AND WHIPPLE, G. H.: Chloroform liver injury increases as protein stores decrease. *Am. J. M. Sc.*, **199**: 204-216, 1940.
185. MILLER, L. L., AND WHIPPLE, G. H.: Liver injury, liver protection, and sulfur metabolism. Methionine protects against chloroform liver injury even when given after anesthesia. *J. Exper. Med.*, **76**: 421-435, 1942.
186. MINOT, A. S.: The mechanism of the hypoglycemia produced by guanidine and carbon tetrachloride poisoning and its relief by calcium medication. *J. Pharmacol. & Exper. Therap.*, **43**: 295-313, 1931.
187. MOISE, T. S., AND SMITH, A. H.: Diet and tissue growth. I. The regeneration of liver tissue on various adequate diets. *J. Exper. Med.*, **40**: 13-23, 1924.
188. MOON, V. H.: Experimental cirrhosis in relation to human cirrhosis. *Arch. Path.*, **18**: 381-424, 1934.
189. MOORE, B.: The causation and prevention of trinitrotoluene (T.N.T.) poisoning. *Medical Res. Com. (Lond.) Special Report Series*, No. 11 pgs. 1-85, 1918. Pg. 75.
190. MORRISON, T. G.: Quantitative study of collagen content in experimental cirrhosis. *J. Exper. Med.*, **85**: 217-225, 1947.

191. MOSIMAN, R. E., AND WHIFFLE, G. H.: Chloroform poisoning. Resistance of the pigeon, frog and terrapin to late chloroform poisoning. *Bull. Johns Hopkins Hosp.*, **23**: 323-325, 1912.
192. MOURLON, F. A. L.: Considérations sur l'emploi du chloroforme dans le traitement de certaines affections chirurgicales. *Strasbourg*, 1853.
193. MOXON, A. L.: Alkali disease or selenium poisoning. *South Dakota Agric. Expt. Sta. Bull.*, No. 311, pgs. 1-91, (May) 1937.
194. MOXON, A. L. AND RHIAN, M.: Selenium Poisoning. *Physiol. Rev.*, **23**: 305-337, 1943.
195. MULLER, J.: Vergleichende Untersuchungen über die narkotische und toxische Wirkung einiger Halogen-Kohlenwasserstoffe. *Arch. exper. Path. u. Pharmacol.*, **109**: 276-294, 1925.
196. NEALE, R. C. AND WINTER, H. C.: Identification of active crystalline substance from liver which protects against liver damage due to chloroform or carbon tetrachloride; and study of related compounds. *J. Pharmacol. & Exper. Therap.*, **62**: 127-148, 1938.
197. NEEFF, J. R., STOKES, J., JR., REINHOLD, J. G., AND LUKENS, F. D. W.: Hepatitis due to the injection of homologous blood products in human volunteers. *J. Clin. Investigation*, **23**: 836-855, 1944.
198. NEUBERGER, A., AND WEBSTER, T. A.: Studies on alcaptonuria. 3. Experimental alcaptonuria in rats. *Biochem. J.*, **41**: 449-457, 1947.
199. NOTHNAGEL, Die fettige Degeneration der Organe bei Aether und Chloroformvergiftung. *Berlin. Klin. Wochenschr.*, **3**: 31-33, 1896.
200. OPTÉ, E. L.: On the relation of combined intoxication and bacterial infection to necrosis of the liver, acute yellow atrophy and cirrhosis. *J. Exper. Med.*, **12**: 367-386, 1910.
201. OPTÉ, E. L., AND ALFORD, L. B.: The influence of diet on hepatic necrosis and toxicity of chloroform. *J. Am. M. A.*, **62**: 895-896, 1914.
202. OPTÉ, E. L., AND ALFORD, L. B.: The influence of diet upon necrosis caused by hepatic and renal poisons. *J. Exper. Med.*, **21**: 1-20, 1915.
203. OPTÉ, E. L., AND ALFORD, L. B.: The influence of diet upon necrosis caused by hepatic and renal poisons. *J. Exper. Med.*, **21**: 21-37, 1915.
204. PAINTER, F. P., AND FRANK, K. W.: On the relationship of selenium to sulfur and nitrogen deposition in cereals. *Am. J. Botany*, **27**: 336-338, 1940.
205. PANTON, P. N., AND BATES, H. E.: Summary of an experimental investigation into T.N.T. poisoning. *Med. Res. Council (Lond.)*, Special Report Series, No. 58, pgs. 62-71, 1921.
206. PATEK, A. J., JR., AND POST, J.: Treatment of cirrhosis of the liver by a nutritious diet and supplements rich in vitamin B complex. *J. Clin. Investigation*, **20**: 481-505, 1941.
207. PETERS, R. A., THOMPSON, R. H. S., KING, A. J., WILLIAMS, D. I., AND NICOL, C. A.: The treatment of post-arsphenamine jaundice with sulphur-containing amino acids. *Quart. J. Med.*, **14**: 35-56, 1945.
208. PHELPS, B. M., AND HU, C. H.: Carbon tetrachloride poisoning. Report of two fatal cases and a series of animal experiments. *J. Am. M. A.*, **82**: 1254-1256, 1924.
209. POLLOCK, L. J., FINKELMAN, I., AND BRIEFF, A. J.: Toxicity of pyridine in man. *Arch. Int. Med.*, **71**: 95-106, 1943.
210. POPPER, H., KOCH WESER, D., AND SZANTO, P. B.: Protective effect of vitamin B₁₂ upon hepatic injury produced by carbon tetrachloride. *Proc. Soc. Exper. Biol. & Med.*, **71**: 688-690, 1949.
211. POST, J., EARLE, D. P., PATEK, A. J., AND VICTOR, J.: Effects of yeast and food intake on experimental carbon tetrachloride cirrhosis of the liver in the rat. *Am. J. Path.*, **18**: 661-673, 1942.
212. QUASTEL, J. H.: Glutathione, isomalic acid and glucose metabolism. *Nature, London*, **131**: 206-206, 1933.
213. RADHAKRISHNA RAO, M. V.: Dietetic hepatic lesions and protein deficiency. *Nature, London*, **161**: 446-447, 1948.
214. RAVDIN, I. S., VARS, H. M., GOLDSCHMIDT, S., AND KLINGENSMITH, L. E.: Anesthesia and liver damage; effect of anesthesia on blood sugar, the liver glycogen, and liver fat. *J. Pharmacol. & Exper. Therap.*, **64**: 111-129, 1938.
215. RAVDIN, I. S., VARS, H. M., AND GOLDSCHMIDT, S.: The non-specificity of suspensions of sodium xanthine in protecting the liver against injury by chloroform, and the probable cause of its action. *J. Clin. Investigation*, **18**: 633-640, 1939.
216. RETTIG, H.: Zur Frage des toxischen Eiweisszerfalls bei der Phosphorvergiftung. *Arch. exper. Path. u. Pharmacol.*, **76**: 345-396, 1914.
217. RHIAN, M., AND MOXON, A. L.: Chronic selenium poisoning in dogs and its prevention by arsenic. *J. Pharmacol. & Exper. Therap.*, **78**: 249-264, 1943.
218. ROBBINS, R. H.: The absorption, distribution and excretion of carbon tetrachloride in dogs under various conditions. *J. Pharmacol. & Exper. Therap.*, **37**: 203-216, 1929.
219. ROSENBAUM, F.: Kohlehydratbestand nach akuten Intoxicationen. *Arch. exper. Path. u. Pharmacol.*, **15**: 450-461, 1882.
220. ROSENFELD, G.: Fettbildung. *Ergebn. d. Physiol.*, **2**: 50-94, 1903.
221. ROSENFELD, G.: Fett und Kohlehydrate. *Berlin Klin. Wochenschr.*, **43**: 978-981, 1906.
222. ROSENFELD, G.: Eiweisskörper und Leberverfettung. *Berlin Klin. Wochenschr.*, **47**: 1268-1272, 1910.
223. ROSENTHAL, S. M.: Some effects of alcohol upon the normal and damaged liver. *J. Pharmacol. & Exper. Therap.*, **38**: 291-301, 1930.
224. SALAMAN, M. H., KING, A. J., WILLIAMS, D. I., AND NICOL, C. S.: Prevention of jaundice resulting from anti-syphilitic treatment. *Lancet*, **2**: 7-11, 1944.
225. SANBURN, W. D., AND WOODYATT, R. T.: Studies on the theory of diabetes. V. A study of narcotic drugs in phlorizin diabetes. *J. Biol. Chem.*, **21**: 1-21, 1915.

226. SCHIFFRIN, A.: Der Einfluss qualitativ verschiedener Ernährungsformen auf die durch Salvarsan hervorgerufenen Lebernekrosen. *Virchows Arch. Path. Anat.*, **287**: 175-202, 1932.
227. SCHNEIDER, H. A.: Selenium in nutrition. *Science*, **83**: 32-34, 1936.
228. SCHULTZ, E. W., HALL, E. M., AND BAKER, H. V.: Repair of the liver following the injection of chloroform into the portal system. *J. Med. Research*, **44**: 207-230, 1924.
229. SCHULTZ, J., AND LEWIS, H. B.: The excretion of volatile selenium compounds after the administration of sodium selenite to white rats. *J. Biol. Chem.*, **133**: 199-207, 1940.
230. SCHULTZ, E. W., AND MARX, A.: Studies on the toxicity of carbon tetrachloride. *Am. J. Trop. Med.*, **4**: 469-482, 1924.
231. SCHWAB, K.: Ueber einen tödlichen, ernährungsbedingten Leberschaden und das Vorkommen von Leberschutzstoffen. *Ztschr. f. physiol. Chem.*, **281**: 101-108, 1944.
232. SCHWAB, K.: Tocopherol als Leberschutzstoff. *Ztschr. f. physiol. Chem.*, **281**: 109-116, 1944.
233. SELLERS, E. A., LUCAS, C. C., AND BEST, C. H.: The lipotropic factors in experimental cirrhosis. *Brit. M. J.*, **1**: 1061-1064, 1948.
234. SELLERS, E. A., ROSEMARY, W. Y., AND LUCAS, C. C.: Lipotropic agents in liver damage produced by selenium of carbon tetrachloride. *Proc. Soc. Exper. Biol. & Med.*, **75**: 118-120, 1950.
235. SHAFER, C. B., CARPENTER, C. P., AND MOSES, C.: An experimental evaluation of methionine in the therapy of liver injury from carbon tetrachloride. *J. Indust. Hyg. & Toxicol.*, **28**: 87-93, 1946.
236. SHAFER, C. B., AND CRITCHFIELD, F. H.: Nitrogen and sulfur content of liver tissue in relation to carbon tetrachloride exposure. *Proc. Soc. Exper. Biol. & Med.*, **59**: 210-211, 1945.
237. SIMMONDS, J. P.: The effect of feeding sugar upon the esterase content of the blood serum and organs in phosphorus poisoning. *J. Exper. Med.*, **28**: 663-679, 1918.
238. SMILLIE, W. G., AND PESSOA, S. B.: Treatment of hookworm disease with carbon tetrachloride. *Am. J. Hyg.*, **3**: 35-45, 1923.
239. SMITH, A. H., AND MOISE, T. S.: Diet and tissue growth. II. The regeneration of liver tissue during nutrition on inadequate diets and fasting. *J. Exper. Med.*, **40**: 209-217, 1924.
240. SMITH, M. I.: The influence of diet on the chronic toxicity of selenium. *Pub. Health Rep.*, **54**: 1441-1452, 1939.
241. SMITH, M. I., LILLIE, R. D., AND STOHLMAN, E. F.: The toxicity and histopathology of some azo compounds as influenced by dietary protein. *Pub. Health Rep.*, **58**: 304-317, 1943.
242. SMITH, M. I., AND STOHLMAN, E. F.: Further observations on the influence of dietary protein in the toxicity of selenium. *J. Pharmacol. & Exper. Therap.*, **70**: 270-278, 1940.
243. SMITH, M. I., WESTFALL, B. B., AND STOHLMAN, E. F.: Experimental Trinitrotoluene poisoning with attempts at detoxification. *J. Indust. Hyg. & Toxicol.*, **25**: 391-395, 1943.
244. SMITH, W. W.: The protective action of cystine and methionine in rats exposed to methyl chloride. *Federation Proc.*, **5**: 97-97, 1946.
245. STEINBURG, B., AND MARTIN, R. A.: Absorption of scar tissue in experimental nodular cirrhosis of the liver. *Arch. Path.*, **41**: 1-10, 1946.
246. STOWELL, R. E., AND LEE, C. S.: Histochemical studies of mouse liver after single feeding of carbon tetrachloride. *Arch. Path.*, **50**: 519-537, 1950.
247. SUGIURA, K., AND RHOADS, C. P.: Experimental liver cancer in rats and its inhibition by rice-bran extract, yeast, and yeast extract. *Cancer Research*, **1**: 3-16, 1941.
248. SVIRRELY, J. L., MONACO, A. R., AND ALFORD, W. C.: The comparative efficiency of various liver function tests in detecting hepatic damage produced in dogs by xylydine. *J. Lab. & Clin. Med.*, **31**: 1133-1143, 1946.
249. SWANSTON, C.: Discussion on trinitrotoluene poisoning. *Proc. Roy. Soc. Med.*, **35**: 553-555, 1942.
250. TANTURI, A., LONCHARICH, J. A., AND BANFI, R. F.: Protective action of sulfanilamide on experimental chloroform hepatitis. *Surg., Gynec. & Obst.*, **64**: 477-480, 1947.
251. THIEM, C., AND FISCHER, P.: Ueber tödliche Nachwirkung des Chloroforms. *Deutsche med. Zeit.*, **10**: 1111-1114, 1889.
252. TROWELL, O. A.: The experimental production of watery vacuolation of the liver. *J. Physiol.*, **105**: 268-297, 1946.
253. UNDERHILL, F. P., AND KAPINSKY, R.: A study of metabolism in chloroform poisoning. *J. Metab. Res.*, **2**: 57-72, 1922.
254. VAN DER SCHUEREN, G.: Essai de Production Expérimentale de Cirrhose à l'Aide de Divers Agents. *Compt. rend. Soc. biol.*, **109**: 982-984, 1932.
255. VOEGTLIN, C., HOOPER, C. W., AND JOHNSON, J. M.: Trinitrotoluene poisoning—its nature, diagnosis, and prevention. *Bull. No. 126, Hyg. Lab., Washington*, pp. 7-182, 1920.
256. VON GLAHN, W. C., AND FLINN, F. B.: The effect of yeast on the incidence of cirrhosis produced by lead arsenate. *Am. J. Path.*, **15**: 771-781, 1939.
257. VON GLAHN, W. C., FLINN, F. B., AND KEIM, W. F.: Effect of certain arsenates on the liver. *Arch. Path.*, **25**: 488-505, 1938.
258. WAKIM, K. G., AND MANN, F. C.: Effect of experimental cirrhosis on the intrasplenic circulation of blood in the intact animal. *Arch. Path.*, **43**: 198-203, 1942.
259. WEGNER, G.: Der Einfluss des Phosphors auf den Organismus. *Virchows Arch. path. Anat.*, **55**: 11-44, 1872.
260. WELLS, H. G.: Chloroform necrosis of the liver. *Arch. Int. Med.*, **1**: 589-601, 1908.
261. WESTROPE, L. L.: The value of a milk diet prior to the use of kharsivan or salvarsan. *Brit. M. J.*, **2**: 456-456, 1916.
262. WHIPPLE, G. H.: Pregnancy and chloroform anesthesia. *J. Exper. Med.*, **15**: 246-248, 1912.
263. WHIPPLE, G. H.: Insusceptibility of pups to chloroform poisoning during the first three weeks of life. *J. Exper. Med.*, **15**: 259-269, 1912.

264. WHIPPLE, G. H., AND SPERRY, J. A.: Chloroform poisoning. Liver necrosis and repair. *Bull. Johns Hopkins Hosp.*, **20**: 278-289, 1909.
265. WHITE, A., AND JACKSON, R. W.: The effect of brombenzene on the utilization of cystine and methionine by the growing rat. *J. Biol. Chem.*, **111**: 507-513, 1935.
266. WHITE, E. G.: The effect of carbon tetrachloride on the liver of the pig, with especial reference to experimental cirrhosis. *J. Path. & Bact.*, **49**: 95-103, 1939.
267. WHITE, J. AND EDWARDS, J. E.: Effect of supplementary methionine or choline plus cystine on the incidence of p-dimethylaminoozobenzene induced hepatic tumors in the rat. *J. Nat. Cancer Inst.*, **3**: 43-59, 1942.
268. WRIGHT, W. H., AND SCHAFER, J. M.: Critical anthelmintic tests of chlorinated alkyl hydrocarbons and a correlation between the anthelmintic efficacy, chemical structure and physical properties. *Am. J. Hyg.*, **16**: 325-428, 1932.
269. WYON, G. A.: Experiments on the toxic effects of trinitrotoluene in animals. *Medical Res. Com. (Lond.)*, Special Report Series, No. 58, pgs. 32-48, 1921.
270. ZAHL, P. A., DRASHER, M. L., AND HUTNER, S. H.: Pharmacological protection against *Salmonella* endotoxin and certain other poisons. *Proc. Soc. Exper. Biol. & Med.*, **63**: 550-555, 1946.
271. DEZALKA, E.: Studies on the effect of chloroform on the liver of rabbits. *Am. J. Path.*, **2**: 167-188, 1926.

EXPERIMENTAL CARDIAC ARRHYTHMIAS AND QUINIDINE-LIKE DRUGS

G. S. DAWES

The Nuffield Institute for Medical Research, University of Oxford, England

The purpose in writing this review has been to examine the methods for producing cardiac arrhythmias in experimental animals; firstly as evidence of the fundamental nature of these disorders of rhythm, and secondly as instruments in the quantitative assay of quinidine-like drugs. The literature on the subject is full of theories about the mechanism of these fascinating arrhythmias, but hard facts are hard to obtain. For in the whole animal there are seemingly endless variables which may influence the result of any experimental procedure.

Probably the most complex method for producing ventricular fibrillation is that which originates from the investigations of Levy (109-111) on the combined action of chloroform and adrenaline on the circulation of the cat. In this method two substances are used, both of which have direct actions upon the heart muscle, and both of which are also capable of causing reflex changes in the circulation. Yet even in such experiments in the whole animal analysis has revealed some of the individual influences which determine whether or not disorders of rhythm shall occur.

The complexities of this problem have presented such a clear challenge to investigators that it is no coincidence that so much original work has followed Levy's brilliant exposition. And it is from this work that much of our knowledge about the physiological variables in arrhythmias is drawn.

THE INFLUENCE OF CHANGES IN THE ARTERIAL BLOOD-PRESSURE

In 1914 Levy (111) wrote that "throughout a long series of experiments with adrenalin the onset of ventricular fibrillation has borne no relation to the height to which the blood pressure has been raised and the supposition of a causal relationship is thereby largely negatived." Many others since his time have made the same observation, but they, like Levy, felt that the point required further investigation.

Levy found that in cats compression of the aorta gave rise to ventricular extrasystoles, attributed to the increased intraventricular tension. But he did not believe that this phenomenon was causally related to the development of ventricular fibrillation under chloroform-adrenaline treatment in the cat, since these rhythmic ventricular extrasystoles were also observed under ether anaesthesia, during which injection of adrenaline did not lead to ventricular arrhythmias. Nevertheless he also found that "when the circulation becomes depressed, as in the case of loss of blood or some other cause" existing cardiac irregularities disappeared; and he concluded that a certain degree of intracardiac tension, about 100 mm. Hg. or over, favoured the development of ventricular extrasystoles and fibrillation. Yet a rise in blood pressure was not necessary for their appearance, as he also showed in an experiment in which the right stellate

ganglion in six cats was stimulated during light chloroform anaesthesia. Ventricular fibrillation resulted in four of these animals, and in two was preceded by no alteration in mean arterial blood pressure. Subsequent investigations have done little to make us modify Levy's original conclusions that while the absolute blood pressure level may influence the outcome of adrenaline injection into such animals, the relative change of pressure which occurs after the injection is not the crucial factor.

In 1938 Shen (177) reopened the question. The introduction of substances like F933 and F883 and the further analysis of the mode of action of yohimbine, which showed that it too possessed the property of suppressing or reversing the pressor action of adrenaline, yielded pharmacological tools for testing Levy's conclusions. In dogs under chloroform, the administration of adrenaline after a sufficient quantity of these adrenolytics had been given to abolish its vaso-pressor action, did not lead to ventricular fibrillation, and Shen concluded that the abrupt and intense rise of blood pressure must normally play a considerable part in its production. The next year however, van Dongen (198) showed that F933 in similar doses in cats and rabbits not only suppressed "heterotopic rhythms" caused by adrenaline and barium chloride, but also raised the resistance of the heart to fibrillation induced by electrical stimulation. These latter actions could hardly be explained by abolition of the pressor response to adrenaline and as a result in 1940 Shen and Marri (179) reconsidered the position after making further observations with a number of other substances, including F1262. Bovet, Fourneau, Tréfouël and Strickler (25) had introduced F1262 by showing that, although closely related chemically to F933, it did not abolish or reverse the pressor action of adrenaline; yet in the rabbit and dog it protected against ventricular fibrillation induced by electrical stimulation, barium chloride and aconitine, and prolonged the refractory period of the frog's heart. Shen and Marri (179) also showed under their own conditions that F1262 prevented ventricular fibrillation caused by administration of benzol and adrenaline. But they injected adrenaline at the same time as F1262, and though as Bovet *et al.* had shown, F1262 does not reverse the pressor action of adrenaline, it does itself cause a large fall of blood pressure. In those instances, therefore, where Shen and Marri obtained protection against benzol-adrenaline fibrillation, the injection had in fact caused a fall in blood pressure and not a rise. As they pointed out, this hardly constituted a fair test of the properties of F1262; on the other hand, if the adrenaline were given 5-60 minutes after the injection of F1262, fibrillation occurred. Since nothing was known of the rate of disappearance of F1262 from the blood stream, no firm conclusions could be drawn. At the same time Shen and Marri reported a further experiment in which it was shown that during benzol inhalation the introduction of 200 γ /kg. adrenaline into the pericardial sac of a dog caused a slow rise of blood pressure, some irregularities but no ventricular fibrillation; later 20 γ /kg. adrenaline intravenously caused a rapid rise of pressure, fibrillation and death. They therefore concluded, considering the evidence then available, that "the joint influence of benzol and adrenaline plus the abrupt arterial hypertension may represent the most potent stimulating

pattern for the production of ventricular fibrillation." Perhaps it would be fair to say that a number of observations had been made consistent with the hypothesis that the rise in pressure after adrenaline-injection was important; on the other hand, it had also quite clearly been demonstrated that the substances involved did have a direct action on the myocardium.

The war then intervened and put a stop to this work. It was started again in the United States with two minor changes. The widespread use of cyclopropane had attracted the attention of investigators to its action upon the myocardium, and cyclopropane rapidly displaced chloroform or benzol as the "sensitising" inhalant anaesthetic in Levy's classical manoeuvre, and dogs were therefore substituted for cats. The reason for using dogs is due to the observation that cyclopropane is more effective than chloroform in the dog (8, 125) and perhaps also to the impression that the reactions of the dog might be more akin to those of man. Secondly, the adrenolytics used before the war were replaced by dibenamine.

Moe, Malton, Rennick and Freyburger (130) and Nickerson and Nomaguchi (142) simultaneously published in 1949 a very interesting analysis of the problem. It was found that in dogs larger doses of dibenamine and priscol were required to protect against cyclopropane-adrenaline induced ventricular irregularities than were required to reverse the pressor response to the same dose of adrenaline. Conclusions of a similar nature were also arrived at in man. On the other hand, ergotamine 0.16 mg./kg. was found to protect against four times the previously effective dose of adrenaline without preventing the pressor effect. Clearly then, the protective action of these adrenolytics was not necessarily related to their ability to abolish the pressor response. Nevertheless, conclusive proof followed that the level of the blood pressure was of importance in relation to this phenomenon. It was found that if dibenamine or a similar blocking agent were given in a dose just adequate to prevent ventricular arrhythmias on administration of adrenaline in presence of cyclopropane, then elevation of the blood pressure, either by means of a regulator in the peripheral circulation or by compression of the aorta, caused the reappearance of ventricular tachycardia or even ventricular fibrillation. A sudden rise of pressure was not necessary; an elevation of the mean pressure was quite effective provided it was of sufficient duration. Nickerson and Nomaguchi (142) emphasize this point, that short periods of hypertension lasting 5-10 seconds lead to few irregularities, and that irregularities persist for up to 10 seconds after a prolonged rise of pressure has been released. Conversely, Moe, Malton, Rennick and Freyburger (130) showed that if the pressor response to adrenaline were limited to only 5-10 mm. Hg. by their peripheral pressure-regulator, then the ventricular tachycardia was prevented. In some experiments eight times the previously effective dose of adrenaline caused no irregularity when the pressure-regulator was in operation. These observations are not in fundamental disagreement with those of Murphy, Crumpton and Meek (136) who found that ventricular tachycardia can still occur when the pressure rise has been virtually prevented by a similar type of stabilizer, since they also found in other dogs treated with cyclopropane and adrenaline that ventricular tachycardia did

not occur unless the blood pressure was allowed to rise. There seems little doubt from the published accounts of these experiments that the part played by the level of the blood pressure varies considerably according to the experimental conditions, the quantity of adrenaline used, perhaps the depth of anaesthesia and the individual animal. If we accept the view that the general level of the blood pressure is only one of many variables in determining whether or not ventricular arrhythmias may ensue, then the various observations are mutually reconcilable. It is quite clear that it is not the crucial factor under all circumstances, and so far it has only been incriminated as a factor at all in cyclopropane-adrenaline arrhythmias.

There are, however, a number of considerations which suggest that it may be profitable to explore the possibility that the blood pressure level is of importance in the development of other forms of ventricular and perhaps of auricular disorders. There is to start with, the often quoted observation by Rothberger and Winterberg (1910) (158) of ventricular premature contractions and a bigeminal rhythm following a rise of systemic arterial pressure due to aortic occlusion in an otherwise normal and untreated animal. This type of observation has often been confirmed, by Levy in 1914 (111) and others since (9, 108, 130) even in the dog heart-lung preparation. While this disturbance is mild compared with ventricular tachycardia, there are perhaps circumstances in which the former might precipitate the latter. There is also at least a suggestion that prolonged rises of intra-auricular pressure may lead to auricular disorders of rhythm. And it has already been reported that increased stretch of the auricles predisposes to arrhythmias induced by aconitine (170). It would therefore be interesting to see whether the general level of the blood pressure affects the "fibrillation threshold" as measured by the method of Wiggers and Wégria (210), or whether a change in intra-auricular pressure alters the ability of electrical stimulation to induce flutter or fibrillation in the auricles.

Finally one may well wonder by what means it is that a rise in systemic arterial pressure influences the ability of adrenaline to induce arrhythmias in a heart under cyclopropane anaesthesia. Nickerson and Nomaguchi (142) have shown that bilateral vagotomy or minimum blocking doses of atropine do not protect against ventricular irregularities, nor does bilateral vagotomy alter the effect of aortic occlusion in inducing irregularities after small protective doses of dibenamine. The effect of a rise in pressure is therefore likely to be direct upon the heart itself. This would be in accord with the observation by Moe *et al.* (130) of a bigeminal rhythm in the dog heart-lung preparation on a sudden elevation of systemic pressure. And one is reminded of the work of Segers (174, 175) in which he found that, in the frog's heart, a rise of intracardiac pressure favoured the appearance of extrasystoles and diminished the "cellular polarisation." Increase or decrease of the degree of distension changed the level of polarisation either so that extrasystoles always occurred or so that they never occurred. In his view the effect of changes in pressure were to be explained by the alteration caused in the degree of polarisation of the cells of the heart.

THE ROLE OF ADRENALINE IN THE DEVELOPMENT OF ARRHYTHMIAS

In the original experiments of Levy in 1911 (109) adrenaline was used in conjunction with chloroform to produce disorders of the rhythm of the ventricles. Since that time it has proved effective after treating a cat or dog with other substances, of which benzol (139, 140) and cyclopropane (126) have been studied most extensively, though many other unsubstituted or halogenated hydrocarbons (35) and iminoazole derivatives (60, 61) possess similar properties. On the other hand, other sympathomimetic amines besides adrenaline can be used in combination with these hydrocarbons to precipitate ventricular arrhythmias. Levy himself used epinine and tyramine (111) and noradrenaline was also shown to be effective by Tournade and Raymond-Hamet (193), Meek (124) and Garb and Chenoweth (65). Meek and his colleagues (124) also found that cobefrin and kephrine were active, but not ephedrine, benzedrine, paredrine, synephrin or neosynephrin. Even simpler aliphatic amines have been shown to possess this type of activity (137). However, of these amines adrenaline has been used far the most widely, and there is no reason to believe that the others differ from it in their mode of action.

Levy (111) also pointed out that adrenaline (to which we should now also add noradrenaline) of endogenous origin may account for those instances of ventricular arrhythmias which occur on the administration of chloroform alone. This was supported by his observation that nicotine, which he knew "accelerated the secretion of the adrenal glands," also could precipitate ventricular fibrillation under chloroform anaesthesia (see also 125). And he emphasized the significance of sympathetic nervous activity still further by experiments in which fatal ventricular fibrillation was precipitated by stimulation of the right stellate ganglion.

Nickerson and Nomaguchi (142) concluded from their observations that the tendency for the heart of an animal exposed to cyclopropane to develop arrhythmias is greatest shortly after adrenaline first reaches it. Thus ventricular fibrillation was precipitated by occlusion of the aorta, and hence a rise in left intraventricular pressure, 15-20 seconds after injection of adrenaline, but not thereafter. Moreover, as they and others have observed, when ventricular fibrillation develops spontaneously in otherwise untreated dogs exposed to chloroform or cyclopropane with adrenaline, it occurs very shortly after the injection of adrenaline, in their experience after only 8 seconds as an average. This would restrict the period of susceptibility from somewhat less than 8 seconds up to 15-20 seconds after injection of adrenaline. Similarly Fawaz (62), although finding it more difficult to produce ventricular fibrillation in the isolated dog heart-lung preparation than in the intact animal, observed that when fibrillation did occur, it did so less than 30 seconds after the injection of adrenaline. Since the peripheral circuit time of the preparation was about one minute, the effect must have been produced by a single passage of adrenaline through the coronary system. And in this instance, as in the experiments of Murphy, Crumpton and Meek (136) quoted above, the fatal ventricular fibrillation occurred in spite of

the fact that there was no substantial alteration in systemic arterial pressure. It would therefore appear that that action of adrenaline which predisposes to or initiates the development of ventricular arrhythmias on intravenous injection is of a fleeting character.

Hoff and Nahum (80, 138) in 1934 gave an account of their views on one of the mechanisms which may bear on this problem. They observed that administration of large doses of adrenaline to cats caused two phenomena, firstly a profound reflex cardio-depressor effect, with bradycardia, slowed auricular conduction and migration of the pacemaker to A-V node and bundle of His, and secondly the appearance of ventricular extrasystoles from one or more foci. When the reflex depressor mechanism was eliminated by cutting the vagi, the ventricular arrhythmias also disappeared. And they drew the conclusion that, for the production of ventricular rhythms, the ventricular rhythmicity must be greater than that of the supraventricular (sinus node or auricular) mechanisms. They had previously observed that benzol "increases the sensitivity" of the ventricle to adrenaline normally present in the body, so that ventricular arrhythmias might occur in the absence of any reduction of the rhythmicity of pacemaker or auricles. And they also found that administration of admittedly large doses of acetyl- β -methylcholine completely suppressed abnormal ventricular rhythms during inhalation of benzol or chloroform. This was attributed to the ability of such a cholinergic drug to reduce to a safe level the increased rhythmicity of the ventricle. Similarly it has been observed that administration of pilocarpine will prevent chloroform-adrenaline syncope (146), perhaps due to the fall of blood pressure. Thus in the first type of experiment reflex vagal stimulation caused by a rise of blood pressure is presumed to reduce the rhythmicity of the auricles more than the ventricles, whereas in the second experiment acetyl- β -methylcholine reduces the rhythmicity of the ventricles more than that of the auricles. (This action of a substance possessing the properties of acetylcholine should not be confused with the well-established ability of such drugs to precipitate arrhythmias under very different circumstances, a phenomenon which is quite distinct and which is discussed elsewhere.) These observations of Nahum and Hoff serve to enlighten the proposition that the distinctive property of adrenaline in precipitating ventricular arrhythmias under appropriate conditions is one of enhancing the rhythmicity of single cells or groups of cells in the ventricular muscle. But since there are many observations of ventricular fibrillation occurring in animals in which both vagi have been cut, the reflex vagal mechanism can only be of subsidiary importance in the mode of action of adrenaline.

Observations by Moe, Harris and Wiggers (129) on the vulnerable period of cardiac muscle suggested to Fastier (60) that "adrenaline syncope might occur when the heart's capacity to respond to a fast rate is so impaired (by treatment with agents like benzene and amarin) that the cardio-acceleration produced by injecting adrenaline results in the stimulus of a new beat coming during the vulnerable period of the preceding cardiac cycle." This was also suggested by the observation that immediately before "adrenaline syncope" the T waves of

the electrocardiogram were interrupted by R waves of succeeding complexes. This analogy with Moe, Harris and Wiggers' experiments is difficult to concede, for the latter had to use electrical stimuli hundreds of times greater than threshold in order to produce ventricular fibrillation. Moreover Fastier found that driving the auricles at an accelerating rate by means of a stimulator was much less effective than a small dose of adrenaline in producing idio-ventricular rhythms in animals treated with amarin or benzol. In fact, after amarin conduction was depressed and the ventricles responded with increasing difficulty to the accelerating auricular discharge. This evidence also points to the probability that the lethal action of adrenaline under these circumstances is due to a direct action on the ventricular muscle. The crucial time has been shown to be that at which the injected adrenaline has just reached the myocardium, and presumably at which the concentration of adrenaline is highest at its site of action. One may also reasonably suppose that, because of the natural vagaries of the coronary circulation, it is the time at which the greatest variations in concentration of adrenaline around the myocardial cells is likely to obtain; one would then also expect the greatest variation in myocardial rhythmicity. The other possible factor in producing such local variations is that of myocardial damage.

THE EFFECTS OF VARIOUS FORMS OF DAMAGE TO THE MYOCARDIUM

In various forms, myocardial damage, provided it is not too severe, has been shown, in some instances very conclusively, to predispose to the generation of ventricular arrhythmias. These include such procedures as exposure to chloroform or barium chloride, anoxaemia, and ligation of the coronary arteries. Sometimes it is said that these "sensitize" the myocardium, in that injection of adrenaline or electrical stimulation may then more readily precipitate fatal ventricular fibrillation. This is perhaps an unhappy use of the term sensitization, which might be taken to imply that the natural functions of the heart are enhanced, whereas the bulk of the evidence points to the opposite conclusion.

1. Anoxia

Although Levy (111) had come to the conclusion that asphyxia only reduced the liability of the heart to ventricular fibrillation caused by the administration of chloroform and adrenaline, Resnik in 1925 (150) considered the possibility that anoxaemia might predispose to auricular fibrillation. In dogs, with both vagi cut and full doses of atropine, he found that when the auricles were stimulated by a faradic current, the early effect of anoxaemia was to predispose to auricular fibrillation. The effect of further anoxaemia was to prevent the development of fibrillation. He also observed that anoxaemia first decreased and later increased the refractory period of auricular tissue, and diminished the rate of fibrillation; but he was understandably cautious in the interpretation of these findings as applied to the genesis of auricular fibrillation. Smith and Wilson (183) used somewhat different conditions. They injected mecholyl into dogs with open chests, and observed that this procedure alone did not induce auricular fibrillation, whereas during anoxaemia caused by stopping the artificial

respiration pump the administration of mecholyl led to its development in 10 out of 19 animals, and that the arrhythmia disappeared when respiratory movements were restored. They made similar observations in heart-lung preparations in which the auricles were not allowed to become distended. And they showed that distension of the auricles also predisposed to auricular fibrillation induced by mecholyl. It would therefore appear that a rise of intra-auricular pressure sometimes has the same effect as a rise of intra-ventricular pressure in determining, under appropriate conditions, the development of arrhythmias. And in the instance cited, anoxaemia had a direct action on auricular muscle, independent of other changes which it causes in the cardiovascular system such as a rise of blood pressure, liberation of adrenaline or nor-adrenaline, or increased sensitivity of the heart to vagal stimulation (77) which also predispose to arrhythmias. There are other observations reported in the literature of the effect of anoxaemia, such as those of Horlick and Surtshin (85) who found that in dogs the production of auricular fibrillation by intravenous injection of large doses of acetylcholine was facilitated by the presence of anaemia due to phenylhydrazine, n-propyl disulphide or haemorrhage. Robbins and Baxter (151) also found that the ventricular arrhythmias developed by pushing cyclopropane anaesthesia in dogs to the point of respiratory arrest could be ascribed in part to anoxaemia, for when this was prevented by artificial respiration the blood cyclopropane concentration could be increased still further without cardiac irregularities. However, in these experiments other variables, such as those considered above, may have altered. This criticism cannot be levelled at the experiments of Wiggers, Wégria and Piñera (211) for they occluded one branch of the left coronary artery for only one or two minutes before testing the fibrillation threshold by rectilinear shocks applied to the ischaemic area of the ventricles during the vulnerable period. A substantial reduction in the strength of current required to induce fibrillation was observed, and this was regarded as one of the principal changes by which myocardial infarction initiates fatal ventricular fibrillation.

These observations, on both auricles and ventricles, taken together constitute a reasonably strong body of evidence to suggest that anoxia of cardiac muscle causes changes which predispose to the development of arrhythmias. They tell us little about the intimate nature of the mechanisms involved. Against the observation of Resnik (150) in the whole dog that anoxaemia initially decreases the refractory period, we have to set that of Wedd (200) that anoxia does not alter the refractory period of the turtle's heart. Nothing is known of the effect of minor degrees of anoxia on single cardiac muscle fibres; and its effect on nerve may or may not be relevant. Yet ischaemia of nerve-endings under certain circumstances may lead to increased excitability and spontaneous discharges (120), a phenomenon which is probably associated with an increase in the negative after-potential (57). There is insufficient evidence to show whether this analogy is too far-fetched.

2. Chloroform, cyclopropane and allied substances

Chloroform, benzol and cyclopropane, to instance three of the substances most widely used in the past to cause ventricular arrhythmias in conjunction

with adrenaline, have been spoken of as "sensitizing" the myocardium. Many investigators have come to the conclusion that these substances cause dilatation and weakening of the beat of the heart (64, 107, 124, 125, 131). In his review of the subject Meek ten years ago (125) concluded that it seemed "impossible to come to any analysis of the chloroform effect on the heart which predisposes it to fibrillation in terms of fundamental muscular physiology." There are still few indications of the type of hypothesis which would explain the surprising discrepancy that while, on the one hand, preliminary treatment with chloroform or cyclopropane is said to "sensitize" the myocardium to adrenaline, no completely convincing evidence has been adduced to show that these substances increase excitability, rhythmicity, the strength of contraction or conduction velocity. On the contrary, the weight of the evidence is strongly in favour of the view that they are "depressant" in all senses of the word.

There are some points of dissimilarity between the actions of chloroform and cyclopropane which may be mentioned:

- i. There seems no doubt that dogs are very much more resistant to chloroform-adrenaline syncope than are cats (8, 125); yet they appear to be very susceptible to cyclopropane-adrenaline syncope, whereas cats are not so susceptible (6).
- ii. As Levy first showed, adrenaline more readily produces fatal ventricular fibrillation in cats under light chloroform anaesthesia than when the anaesthesia is deeper. Similarly Tournade, Malméjac and Djourné (192) observed that faradic stimulation of the ventricles of the dog produced fibrillation in light but not in deep chloroform anaesthesia. On the other hand, Meek, Hathaway and Orth (126) showed that increasing depth of cyclopropane anaesthesia was accompanied by increasing susceptibility to the action of adrenaline in precipitating ventricular fibrillation. More recently Stutzman and Allen (186) found that *prolonged* administration of cyclopropane much reduced or even abolished the ability of large doses of adrenaline to produce ventricular tachycardia or fibrillation; however, it also reduced or reversed the pressor response.

These differences raise the question as to whether chloroform and cyclopropane really are working in precisely the same way, or whether perhaps one of them has some additional property which might account for the discrepancy. As Meek suggests, the effect of deep chloroform anaesthesia is not difficult to understand, and we are here dealing most probably with a simple toxic effect. In such deep anaesthesia all functions of the heart may be so far depressed that it is no longer susceptible to the peculiarly lethal effect of injected adrenaline; in fact, under such circumstances adrenaline is an effective restorative of a rapidly succumbing circulation (74, 192). Meek, Hathaway and Orth's experiments on cyclopropane quoted above included some observations under anaesthesia of a sufficient depth to arrest the intercostal muscles, and those of Robbins and Baxter (151) went still further; when the asphyxia was removed by artificial ventilation, even more cyclopropane had to be administered before spontaneous arrhythmias returned. Thienes, Greeley and Guedel (191) found that high concentrations of cyclopropane, during artificial respiration, abolish or minimize the incidence of spontaneous arrhythmias, though they did not then

examine the efficacy of adrenaline. On the other hand, Lee, Orth, Wangeman and Meek (107) found under similar conditions, using a larger series of dogs, that the incidence and severity of spontaneous arrhythmias increased as the concentration of cyclopropane was raised. Thus, bearing in mind the difficulty of interpreting many experiments in which the effect of systemic blood pressure changes cannot be assessed, there is as yet no convincing evidence that a high concentration of cyclopropane has any protective action in the intact animal against either spontaneous or adrenaline-induced arrhythmias.

There are many similarities in the actions of these anaesthetics. For instance in a preparation of the cat auricle at 30°C, Acierno and DiPalma (2) have recently shown that while ether, chloroform and cyclopropane depressed excitability and contractility, the refractory period was shortened by all three in direct relation to the concentration. This observation does not explain the greater incidence of ventricular irregularities under chloroform and cyclopropane as compared with ether, and if reduction in the refractory period is the crucial factor, this is difficult to reconcile with the observation of Greisheimer *et al.* (73) that simultaneous administration of ether reduces the incidence of arrhythmias under cyclopropane-adrenaline treatment. It is possible that in the whole animal changes in the refractory period produced by these anaesthetics may be of practical importance though it is certainly not the only determinant of their action.

There is another way in which chloroform, benzol or cyclopropane directly influence the heart. It has often been envisaged that they may cause local islands of damage, to a more severe degree than that suffered by adjacent tissue, and that local blocks may ensue under unfavourable conditions. An experimental example of this type of block and its effect on cardiac muscle has been provided by García Ramos (66). Garb² and Chenoweth (65) have shown that benzol or chloroform decrease the electrical excitability of an isolated cat papillary muscle to a much greater extent than ether (compare 2) and this might explain the differences between their actions. There is a further possibility along the same lines of argument. The experiments of Hoff and Nahum (80) as already described led to the view that, for the production of ventricular rhythms, the ventricular rhythmicity (or automaticity) must be greater than that of the supra-ventricular (sinus node or atricular) mechanisms. If we were to suppose that the action of these anaesthetics, which we know depress certain cardiac functions, was greater on the auricles than upon the ventricles, one can readily visualize under the influence of adrenaline the origin of the simpler forms of ventricular arrhythmia. There is no direct evidence to support this hypothesis but it should not be too difficult to put to experimental test.

The possibility that these anaesthetics may have a reflex effect upon the heart has been extensively explored. In 1912 Elliott (56) showed that anaesthesia with chloroform or ether was followed by reduction in the quantity of adrenalin stored in the suprarenal glands. Ether anaesthesia was shown to cause peripheral vasoconstriction and relaxation of the stomach and intestines (127), sweating in the innervated cat's fore-paw but not on the denervated side

(29), contraction of the spleen, inhibition of the virgin cat uterus and rise in heart rate (Bhatia and Burn, 19). The latter also found that these effects were not seen in a fully pithed animal, and that chloroform, which Miller (127) had shown also relaxed the intestines, acted in the same way as ether upon the spleen in their preparations. Samaan (161), by section of the nerve pathways involved, also came to the conclusion that ether increases the heart rate by reflex mechanisms involving both the augmentation of cardio-sympathetic impulses and the liberation of sympathomimetic substances via the splanchnics and abdominal sympathetic chains. Johnson (94) similarly found that ether anaesthesia no longer produced a rise in blood sugar in rabbits after bilateral adrenalectomy.

For cyclopropane, however, the evidence is more fragmentary. Barman (14) observed no increase in adrenaline secretion from the adrenals of dogs during cross-perfusion experiments. Youmans, Wangeman, Griswold and Karstens (214) showed that there was only a small rise in blood sugar, of up to 20 mg. per cent, under cyclopropane anaesthesia in dogs, and the cause of this rise was not determined. On the whole, the balance of evidence suggests that there is some increase in heart rate (125) though the mechanism has still not been adequately analysed.

On the other hand, there is a considerable body of evidence, though for various reasons not always conclusive, that suggests that some form of reflex mechanism may under particular conditions influence the outcome of chloroform- or cyclopropane-adrenaline treatment in the whole animal. Some of this has been discussed by Meek (125), who has made the good point that some of the earlier work may be misleading, since it was based on only a limited range of dosages, and that full account was rarely taken of the differences between species, or of the need for a rigorous control of the depth of anaesthesia and of the concentration and duration of adrenaline injections. The introduction by Meek, Hathaway and Orth (126) of a strictly standardised technique marks an important step forward in the analysis of this phenomenon, though the necessity for taking some account of the mean blood pressure obtaining at the time of the test was not then appreciated. These considerations complicate assessment of the available evidence.

Beattie, Brow and Long (16) showed that extrasystoles induced by chloroform anaesthesia in cats could be abolished by section of the hypothalamus, and that under chloroform anaesthesia stimulation of the posterior part of the lateral wall of the third ventricle produced extrasystoles. It seemed very reasonable to ascribe these observations to stimulation of the sympathetic outflow to the heart, since the arrhythmia disappeared after removal of the stellate ganglia or the sympathetic fibres supplying them, or to stimulation of other sympathetic pathways controlling the secretion of sympathetic amines which might be liberated in the blood. Such a conclusion concurs with Levy's original ideas (110, 111). But when we turn to the production of more serious forms of ventricular arrhythmia by chloroform or cyclopropane and adrenaline the position is confusing. On the one hand, there is no doubt that ventricular fibrillation may ensue in an

animal with a denervated heart (13, 110), or in a heart-lung preparation (62), yet various authors have reported that removal of the lumbar sympathetic chains (146), denervation of the carotid sinuses and section of the afferent nerve-fibres from the aorta (24), decerebration by a bloodless method or thoracic sympathectomy (7), partial abdominal evisceration, partial abdominal denervation or bilateral adrenalectomy (188), or removal of stellate ganglia (5) and adrenals (81) affords a measure of protection. On the other hand Rennick, Pardo, Gruhzt and Moe (149) concluded that the minor degree of protection afforded by thoracic sympathectomy was due to a reduction in the peak pressor response to adrenaline. When this was restored by infusion of blood the original sensitivity returned. The simplest explanation of this confusing wealth of scattered material, of which this is almost certainly not a complete summary, is that under the particular experimental conditions used, the removal of even a small part of the continuous secretion of sympathomimetic amines, or even the operative procedure alone is sufficient to upset the very delicate balance of the numerous factors which influence the outcome, so that the test dose of adrenaline is no longer effective in precipitating ventricular fibrillation. That this is the likeliest explanation is supported by those authors who have observed that, after their operative interference, adrenaline may yet be effective provided the dose administered is sufficiently increased. And this observation also agrees with Fawaz's comment (62) that ventricular fibrillation under cyclopropane or chloroform anaesthesia is more difficult to obtain in the dog heart-lung preparation than in the intact animal. There seems, then, some reason to believe that endogenous reflex secretion of adrenaline or noradrenaline may influence the outcome; and it is still possible that certain anaesthetics might accelerate this secretion in some way yet to be determined, perhaps by sensitization of afferent sensory pathways such as that which Whitteridge and Bülbring (207) described for the pulmonary stretch fibres.

This is not, however, the whole of the story. Ether anaesthesia has been very adequately demonstrated to cause just that effect in enhancing peripheral sympathetic discharges, which is now very tentatively postulated to play a part in the development of other anaesthetic-adrenaline ventricular arrhythmias. But ether is the least efficient of these anaesthetics in causing such disturbances; there is very reasonable doubt whether it ever does so. At most, therefore, this particular factor of increased sympathetic activity is a subsidiary one, to be placed on the same level perhaps as the rise of blood pressure in the mode of action of adrenaline in relation to this problem. Whether such substances as cyclopropane may also, reflexly or centrally, affect the parasympathomimetic control of the heart, there is only little evidence to suggest (152).

3. Barium chloride and other substances

The question of the mode of action of barium chloride in initiating ventricular arrhythmias has been reserved for separate consideration, because it raises in a particularly clear form certain ideas which might also have been discussed in relation to other substances which, alone or in combination, have this property.

Rothberger and Winterberg (159), Levy (111), and others (123) have shown it may cause ventricular extrasystoles, tachycardia or fibrillation. Barium chloride, injected into cats, dogs and rabbits has also been used to induce ventricular arrhythmias, with a view to studying their prevention by substances which might be expected to have an action like that of quinidine (20, 21, 25, 196, 198). Smith, Winkler and Hoff (184) showed that intravenous injection causes a sharp rise of blood pressure at the same time as extrasystoles occur. But this rise of blood pressure probably is not in itself responsible for the dramatic effect upon the heart. Rothberger and Sachs (157) confirmed the observations of earlier workers (105) that it initiated automatic activity in quiescent strips of mammalian left auricle and Deutsch and Lumdin (44) have shown that minute amounts of barium have the same action on small muscle bundles from the frog's ventricle. These observations are of particular interest in view of the well-documented ability of barium to initiate rhythmic activity in nerve. Feng (63) and Dun and Feng (52) showed that conduction of a volley of impulses may be followed by repetitive discharges in barium treated nerve. Occasionally spontaneous discharges also occurred. A detailed analysis of this action was carried out by Lorente de N6 and Feng (116) who found that barium caused depolarisation and a considerable increase in the after-potentials. No doubt these findings account for the observation that in intact cats barium in small doses excites, and in large doses inhibits, the discharge from the superior cervical ganglion (11).

To digress for a moment, Hazard & Quinquaud (76) have shown that the rise of blood pressure caused by intravenous injection of barium chloride is abolished by yohimbine, which is well known as a sympathicolytic. This observation, in conjunction with those detailed above, makes it seem possible that the blood pressure rise may well be due to enhanced sympathetic outflow, and liberation of adrenaline and noradrenaline in the heart from the adrenals and elsewhere. If this were so we have to envisage the possibility that in the whole animal the action of barium chloride upon the heart may be complicated by not only the rise of systemic arterial pressure, but also the liberation of other substances which may have in themselves an action predisposing to the development of arrhythmias. It is interesting that these considerations, which have arisen first in connection with substances such as chloroform and cyclopropane, should also apply to barium. There is little doubt that they are of very much more general application, and, for instance, must be borne in mind in any discussion of the mode of action of the veratrum alkaloids in precipitating cardiac arrhythmias in the intact animal (101).

Of more importance than this is the general proposition suggested by the somewhat analogous action of barium chloride upon nerve and heart muscle. It is pertinent to ask whether there are any other such substances which have properties of the same nature, and the example of the veratrum alkaloids immediately suggests itself. These alkaloids have been very widely used, usually in an impure form, for the study of changes produced in nerve, where they may cause under appropriate conditions repetitive discharges in response to a single stimulus, and, what was very striking when it was first discovered, a great increase in

the negative after-potential (71, 101), with a parallel increase in the supernormal period. These substances excite or sensitize repetitive discharges from peripheral sensory receptors in minute concentrations. In low concentrations they have a positive inotropic effect upon the heart similar to that of the digitalis glycosides and in higher concentrations cause arrhythmias of all types and finally ventricular fibrillation. This analogy between the actions of veratrine upon nerve and heart muscle in causing supernormality has been pointed out before (70). Recently Scherf and Chick (167) described in detail the arrhythmias generated by the topical application to the dog's ventricle of the crude alkaloidal mixture veratrine, or the pure alkaloids veratridine and cevadine in strong concentrations. They concluded that the mechanism of abnormal impulse formation from an ectopic centre was not closely related to the veratrine effect on nerve and muscle; but this distinction appears somewhat arbitrary in that it rests chiefly on the definition of where the first impulse which initiates the repetitive discharge comes from.

Aconitine may prove to be a substance of the same nature. Its toxic properties have been appreciated, like those of veratrine, for centuries. In 1929 Scherf (163) gave a modern account of its action upon the heart, and emphasized its effect in increasing excitability; more recently he (164, 169) produced additional evidence by local application that its action upon cardiac muscle is a direct one. It has a similar effect upon isolated preparations (86, 157), as many earlier workers have shown (22), and like barium has been used quite extensively to induce ventricular arrhythmias, to prevent which ions such as magnesium (87) or drugs such as F1262 (25), α -fagarine or procaine (195) may be tested. The introduction by Scherf of a method of causing auricular arrhythmias by local application of aconitine has caused wider interest in this drug, which has therefore been investigated more thoroughly than for several decades. About the details of its action upon the fundamental properties of mammalian cardiac muscle remarkably little is known. It would be highly desirable to know how this remarkable substance affects not only excitability, power of contraction, and rhythmicity of cardiac muscle, but also what it does to conduction velocity and refractory period. There is a recent report by Charlier and Klutz (34) that two sympathomimetic amines are able to prevent the ventricular tachycardia caused by local application of aconitine. The implications of this observation on its mode of action have still to be examined. Segers (174, 175) observed that it had the same action on late negativity in the isolated frog's heart as did veratrine and barium. And Graham and Gasser (71) found that it also somewhat increased the height and duration of the negative after-potential in isolated nerve, though this action was much less dramatic than that of veratrine. There is also circumstantial evidence that aconitine may be able to provoke repetitive discharges from nerve. Like the veratrum alkaloids, application to the mouth or tongue, or indeed elsewhere in the body, first arouses a sensation of heat, tingling and then succeeding numbness. Strong concentrations similarly first stimulate and later paralyse peripheral motor nerves, the stimulation being manifested by fibrillary twitching. It would certainly be desirable to confirm this hypothesis by direct methods.

The effects of these three substances, barium chloride, veratrum and aconitine, upon nerve and cardiac muscle make one suspect that there may be a common factor in their actions. Since in isolated nerve the supernormal phase has been related to the negative after-potential, it seems worth while enquiring as to the existence of supernormality during the recovery phase of excitability in cardiac muscle.

SUPERNORMALITY AND MEMBRANE POTENTIALS

In 1919-20 Adrian (3, 4) demonstrated the existence of a supernormal phase of excitability during the recovery period in frog heart muscle and he related this to the presence of an acid environment. Wastl (199) made similar observations in fatigued preparations of the frog's heart. In 1934 Eccles and Hoff (54) showed that in some instances the recovery curve of excitability of the "pacemaker" of the cat's heart also passed the previous threshold and thus gave rise to "supernormality," though this was not related directly to the genesis of the next normal contraction. It would appear from the published accounts that a supernormal phase of excitability is not always nor very easily seen in cardiac muscle, unless it has been exposed to some noxious agent. In the untreated isolated auricle of the guinea pig at 37°C it has only been observed on three or four occasions, and then it appeared only for a few minutes, despite intensive search on a great number of preparations (41). Hoff and Nahum (82) showed that supernormality could be observed in the ventricle of cats under barbiturate anaesthesia, but not so readily after decerebration. They point out that this supernormal period fell during a period of continued electrical activity as shown by the electrocardiogram, and the cycle of excitability was determined at a number of different points on the surface of the ventricle. They suggested that these facts, considered in connection with the association of the supernormal period in nerve with the negative after-potential, imply a similar relationship in the mammalian ventricle. In 1941 Segers (174, 175) made a series of interesting observations on the isolated frog's heart, and produced evidence of the association of supernormality with the negative after-potential and of subnormality with an enhanced positive after-potential. He concluded that extrasystolic arrhythmias developed under the influence of all agents which augment the negative after-potential, such as an increase in the calcium concentration of the perfusion fluid, and the administration of aconitine, veratrine, adrenaline, strophanthin and digitalis; rhythmic stimulation of the cardiac muscle at frequent intervals also led to the successive accumulation of slow negative potential changes. This was also increased by distension of the heart and by appropriate polarisation. On the other hand agents which increased the positive after-potential, such as a rise in the potassium concentration of the perfusion fluid, acetylcholine, quinidine and cocaine, caused a diminution in excitability and inhibition of spontaneous activity. The method which Segers used for these measurements was to record the potential changes from two points on the surface of the ventricle, or from between a point on the ventricle and the perfusion fluid in the interior of the heart; in either instance one calomel half-cell attached to the ventricle was filled with saturated KCl, in order that

the responses should be unipolar. The recent publication of methods of obtaining membrane action potentials from single cardiac fibres of the frog heart *in situ* (212, 213), or from "false tendons" or Purkinje fibres of the dog and kid heart (50) makes it highly desirable that these observations of Segers should be repeated on such preparations, in order to obtain unequivocal records of these changes.

There does seem a real chance of unifying many of the factors which are concerned in the aetiology of arrhythmias into a common framework, by studying their actions on such isolated preparations. It is not to be supposed, however, that experiments of this type will alone solve all the problems with which we are concerned for there is little doubt that the syncytial nature of cardiac muscle in itself contributes greatly to the complexities of the problem. But without the evidence as to what drugs and other procedures do to single cardiac muscle fibres it is difficult to believe that much further progress will be made.

There is one further point which may now be made regarding the mode of action of chloroform and cyclopropane in predisposing to the development of cardiac arrhythmias under the influence of adrenaline. Apart from the various mechanisms which have already been discussed, there is the possibility of a direct action upon the myocardium, of a similar nature to that suggested by Segers to account for the action of barium chloride, aconitine etc., as explained above.

THE ECTOPIC FOCUS AND CIRCUS MOVEMENT THEORIES IN THEIR RELATION TO THE REFRACTORY PERIOD

In any general discussion of the initiation of cardiac arrhythmias, the relative merits of the ectopic focus and circus movement theories, in connection with both auricular and ventricular disorders, is bound to arise. This appears to be an appropriate point at which to consider them, because it must be obvious that the possibility of repetitive discharges originating from a single cell, by virtue of such changes as Segers described, implies an ectopic focus as the origin of the disturbance. The term ectopic focus, which has been so widely used in such discussions, does not necessarily mean that the discharge is arising from one single cell. There are several possibilities; an ectopic focus might consist of: i. a single cell; ii. a small group of cells, of which first one and then another becomes the pacemaker; iii. a small group of cells in which a circus movement is perpetuated. No comment is required on the first and second hypotheses; for the sake of simplicity the term unicellular ectopic focus will be used for either. To the third it may be objected that under the observed conditions of refractory period and conduction velocity obtaining in a living heart, the circuit would have to be so large that it would constitute an obvious circus movement, and not a small focus of irregularities located to a finite point in the cardiac muscle. This point of view might be taken to be substantiated by the calculations of Rosenblueth and Wiener (156) on the minimum size of the circuit required for maintenance of the disturbance in a dog's heart and the practical demonstration by Rosenblueth and García Ramos (155) that when the orifice in the auricles was

sufficiently enlarged by crush injury, a maintained circus movement could readily be established. In the normal heart this argument is undoubtedly valid, but in the heart treated with cyclopropane, barium chloride or aconitine, there are not sufficient data to judge. If in any region of damage conduction velocity is reduced, and if in particular transmission is very greatly or entirely impeded across the centre of that region, it is a theoretical possibility that, owing to the syncytial construction of cardiac muscle, a circus movement of very much smaller dimensions might be established. The difficulties of deciding between such possibilities in the living animal are so great that investigators have wisely been content not to enquire too closely into the meaning of the term "ectopic focus"; but from the point of view of the search for substances which may prevent the development of disorders of rhythm, this differentiation may well be of importance.

There is a further theoretical point to consider about the development of a rhythmic disturbance in a unicellular ectopic focus. It has been repeatedly observed that when a supernormal phase of excitability in cardiac muscle is encountered, it occurs early in diastole, just after the beginning of the relative refractory period, and therefore presumably close to that time at which repolarisation of the membrane is completed. If therefore a second discharge arises because of the existence of this supernormality, it must arise at this instant, and diastole will be cut short. This is what Segers (176) describes as "*le battement auto-entretenu du coeur*." If on the other hand a unicellular ectopic focus has become pacemaker to the heart, not because it fires off during the supernormal period of excitability, but because its normal automatic rhythm has been much enhanced, then the relative refractory period and diastole will not be curtailed. In the latter instance it is possible to conceive circumstances under which the frequency of the heart may rise smoothly from that which obtained under the normal influence of the sinus node to a higher value imposed by the ectopic focus. But if the activity of the ectopic focus is determined by the sudden appearance of supernormality, then one would expect a large stepwise increase in frequency as the ectopic focus became active. In the latter instance a decrease of the absolute refractory period, which would presumably be accompanied by an equivalent shift in the position of the supernormal period nearer to the spike, the point at which membrane depolarisation began, would then be accompanied by a substantial increase in discharge frequency. On the other hand, there is no reason to relate alterations in the refractory period to changes in the normal autogenous rhythm of the cardiac muscle. Perhaps an example will make the position clear. It has been known for very many years that acetylcholine causes a substantial decrease in the absolute refractory period, yet it slows the normal rhythm of the heart. The action of acetylcholine upon a unicellular ectopic focus beating by virtue of its inherent rhythm would therefore be to slow it, but if this ectopic focus were discharging according to a rhythm determined by a supernormal period occurring immediately following the absolute refractory period, then we should expect its discharge rate to be increased. This concept might well account for the ability of the vagus to increase the rate of auricular

flutter, while it stops stimulus formation during sinus rhythm or other auricular tachycardias, and Garrey in 1924 (68) considered this proposition.

An agent which reduces the absolute refractory period would also be expected to increase the frequency of a circus movement, and that was Lewis, Drury and Bulger's explanation of their observation that vagal stimulation increases the frequency of the oscillations in auricular fibrillation (112, 113). There are very many references in the literature to the ability of vagal stimulation, acetylcholine or substances closely related to it to provoke disorders of rhythm. Both in animals (41, 83, 91, 144, 153, 183) and in man (15, 36, 141) these have caused auricular fibrillation. Scherf *et al.* (169) observed that vagal stimulation increased the rate of auricular flutter induced by local application of aconitine to the auricle. And in the isolated auricle in which repetitive discharges have been initiated by appropriate stimulation early in the relative refractory period (40), acetylcholine greatly increases the frequency of discharge. When the refractory period is reduced by the presence of acetylcholine, repetitive discharges are also very much easier to initiate, and last for very much longer. Similarly Brown and Acheson (27) observed that stimulation of the vagus or injection of acetylcholine increased the rate of auricular flutter in the dog, in which an auricular circus movement had become established according to the technique of Rosenblueth and García Ramos (155). Indeed with adequate doses of acetylcholine auricular fibrillation occurred. It is difficult to believe that all these observations on the effect of vagal stimulation or the injection of cholinergic substances on the initiation, maintenance and increase of frequency of auricular arrhythmias can be explained other than by a reduction of refractory period. For such procedures all decrease the automatic rhythm of the heart. However, as explained above, a reduction of refractory period might increase the rate of discharge both of a circus movement, or of a unicellular ectopic focus *dependent for its rhythm on supernormality and the refractory period*. These observations then cannot help us to decide between these two alternative mechanisms.

To digress, Scherf *et al.* (169) did consider the possibility that vagal stimulation might have a sympathomimetic action upon the heart, by way of the accelerator fibres which run in the vagus. Indeed it has been shown that an adrenaline-like substance is liberated on injection of acetylcholine (84). This however, appears to be due to a nicotine-like action of acetylcholine and is not paralysed by atropine, whereas the effect of acetylcholine in provoking auricular fibrillation is abolished by atropine (83, 166).

The next type of experiment in which we might hope to obtain information about the nature of the arrhythmia is that in which electrical stimulation of various kinds is used to start the disturbance, and this is a subject which deserves separate consideration.

ELECTRICAL STIMULATION IN THE INITIATION OF ARRHYTHMIAS

Faradisation of the auricles or ventricles has been known for many years, to start flutter or fibrillation. According to the species and condition of the animal, faradisation of the auricles may cause a transient or continuing arrhythmia.

Rectangular stimuli of constant form are just as effective as the unpredictable output of an induction coil, but no systematic analysis appears to have been made of the relationship between frequency and strength of the threshold stimulus required to start flutter on the one hand, and the refractory period prevailing in the muscle on the other hand.

A single shock delivered at an appropriate instant in the cardiac cycle will also initiate arrhythmias, and this type of excitation is more readily analysed. In 1921 de Boer (23) showed that fibrillation of the ventricle of the frog's heart could be induced by a single induction shock applied directly after the end of the refractory period; he also believed that a bad metabolic condition of the heart favoured fibrillation. In 1930 Andrus and Carter (12) showed that during vagal stimulation, a single induction shock applied to the dog's auricle *in vivo*, soon after the end of the refractory period, frequently produced auricular fibrillation. It was later shown that a single D.C. shock of 10-30 msecs. duration applied to the dog's ventricle during the T wave induced ventricular fibrillation, and the work of Wiggers and Wégria (205, 209, 210) put this method on a sound foundation as a technique for determining the "fibrillation threshold" of ventricular muscle and the action of various substances on it.

Wiggers and his colleagues state that in order to elicit multiple extrasystoles or fibrillation, current strengths are necessary (10-20 ma) several hundred-fold stronger than that required for threshold shocks during diastole (129). Ventricular fibrillation initiated in this way starts with a series of three to six large bizarre electrical complexes which recur at progressively decreasing intervals and change their form in successive beats (208). It was clear that the effective stimulus fell near the end of the absolute refractory phase, and it was suggested that this gave rise to a "rhythmic centre from which several impulses are discharged at an accelerating rate." And again, "the progressive decrease in refractory periods associated with the accelerating rate of responses, combined with delay in conduction, furnish ideal conditions for re-entry of impulses, *but only after the second, third or fourth truly premature beats have run their course*" (129). The conclusion that the initial accelerating discharges originated from a single rhythmic centre was supported by the observation that the order of excitation on the surface and interior of the ventricle (as measured by three electrodes close to the point of stimulation) during these initial responses remained the same, and that there was a period between beats when no area was excited. Various reservations must be made before agreeing with this conclusion; it is not clear to what extent the strong and presumably widespread tissue polarisation which results in the neighbourhood of the stimulating electrode, as a result of the enormous shock applied, may subsequently alter conduction velocity and refractory period below that electrode. If these variables were substantially modified, a very much smaller circus movement might have been generated than that which the authors designed their experiment to detect. It is also arguable whether three electrodes distributed according to their pictures across the ventricle were sufficient. To determine the sequence of events in the isolated auricle very many more test points were required (41). And it is pertinent to suggest that the application of

so large a current pulse when effective, may well depolarize simultaneously a considerable area of muscle, and thus create conditions in which a small local circus movement may arise. However, since the authors have been at pains to exclude a small circus movement, within the limits of their experimental methods, the possibility that a "unicellular ectopic focus" is responsible must be considered. And since to be effective the stimulus must be applied during the "vulnerable period," which appears to be very early in the relative refractory period, it seems at first sight that a unicellular ectopic focus might be discharging during supernormality. But before reaching this conclusion there is another feature of this procedure which deserves close attention, that is, the necessity for using a current strength several hundred-fold greater than that necessary for threshold excitation during diastole, after the end of the relative refractory period. It hardly seems likely that such an excessive current strength would be required if supernormality were a regular feature of the dog's ventricle. Harris and Moe (75) could indeed find no evidence of supernormality. Moe, Harris and Wiggers (129) concluded that the tissue polarisation of such a strong brief shock is so great that it persists until early diastole, and is therefore effective even if delivered at a time before this, before the end of the T wave and therefore presumably before repolarisation is complete. These calculations were based on measurements taken from the external surface of the heart, and the value of the tissue polarisation is therefore likely to be much underestimated because of the shunt afforded by surrounding tissue. It would be interesting to know what effect stimuli of this magnitude have on the membrane potential of a single fibre; observations such as those of Weidman (206) seem likely to illuminate this aspect of the subject. It is possible that the residual polarisation which these workers have postulated as the effective stimulus for the first beat of the "rhythmic centre" is also responsible in part for the initiation of subsequent beats. Harris and Moe (75) have examined the effects of anodal and cathodal polarisation in causing arrhythmias in the dog's heart. Segers (176) also has shown that appropriate polarisation is in itself sufficient to initiate automatic discharges from an otherwise quiescent piece of cardiac muscle, and he relates their origin to the appearance of supernormality. It is certainly true that the crucial initial discharges, which recur at progressively decreasing intervals after the application of an effective D.C. shock to a dog's ventricle, and which precede the development of fibrillation, are of a frequency very much greater than that of the natural rhythm of the untreated heart, and the intervals between these discharges very roughly approximate to the expected value of the refractory period. The phenomenon of the acceleration of these discharges might be related to the well-known observation that increasing the frequency of stimulation of cardiac muscle reduces the refractory period (75, 128). As has been pointed out above, evidence of this type associates the mechanism responsible for the repetitive discharges with the refractory period but it does not distinguish between a unicellular ectopic focus hypothesis and a small circus movement hypothesis.

In isolated preparations the opportunity for accurate measurement of the time relations between the arrival of the excitatory process at various points in

the tissue is greatly increased. It was observed by Segers (176) that rhythmic stimulation of a quiescent auricle needed to exceed a certain critical frequency in order that the auricle should continue to show repetitive discharges after the stimulation was stopped. These repetitive discharges continued for a short length of time and then abruptly ceased. They were related by Segers to the presence of supernormality. During investigations on the refractory period of isolated auricle Dawes and Vane (40) made somewhat analogous observations. It was found that a single shock applied very early in the relative refractory period is sufficient to cause a burst of repetitive discharges, at a frequency much greater than the normal rate of auricular beat; the intervals between these discharges were approximately constant and slightly greater than the value of the absolute refractory period just determined. When first observed it was only possible to obtain a few such discharges, but since that time the phenomenon has been found to continue for an hour or more under favourable conditions. These include any change, such as increase of temperature or addition of acetylcholine, which decreases the refractory period (Cf. 47). It was also found that rhythmic stimulation of a quiescent auricle would initiate the repetitive discharges, provided that the frequency of stimulation approximated to or somewhat exceeded that at which the interval between stimuli equalled the absolute refractory period. This then is probably the explanation of Segers' observation referred to above, and agrees with recognition by Wiggers and others of the necessity for an effective stimulus to fall during the "vulnerable period" in order to produce ventricular fibrillation, whether it be a single D.C. shock or alternating current.

This phenomenon of repetitive discharges at high frequency from auricular muscle was found to occur in small strips of muscle free, so far as was ascertainable, from a central orifice or area of damaged tissue, so small that it was thought hardly possible for a circus movement to exist (40). But since that time, on more than one occasion, sufficient evidence has been accumulated by recording from a large number of places on a single auricular strip during one unchanging repetitive discharge lasting half an hour or more, to convince the observers that a circus movement existed in a small section of the muscle. This does not exclude the possibility that in other experiments the disturbance may have originated from a point focus, but it does suggest that under favourable conditions a circus movement may exist in smaller pieces of tissue than had been supposed. And incidentally, in conformity with the observations of Mines (128) and Garrey (67, 68), it was found that the discharge could be stopped by a single properly-timed stimulus.

THE UNICELLULAR ECTOPIC FOCUS THEORY

This seems an appropriate point at which to discuss the direct experimental evidence for the existence of what has been called a unicellular ectopic focus. That thought to be produced by a single D.C. shock in the mammalian ventricle has already been considered above.

In the dog's auricle Scherf found that topical application of aconitine as a

subepicardial injection of a dilute solution, or by the use of a few crystals on the exposed surface, caused auricular tachycardia or fibrillation (164, 168); cooling this small area stopped the arrhythmia, though it reappeared almost at once on warming. He and his colleagues found it difficult to believe that a small circus movement could be involved because the flutter or fibrillation returned so readily. Later they also observed that stretching the wall of the auricle increased the rate of discharge, and converted tachycardia to transient fibrillation (170). They drew analogies between their observation of the effect of stretch on impulse formation in the mammalian auricle and in other tissues, sensory and motor nerves and skeletal muscle. They believed that the mechanical devices which they used for causing stretch influenced only the formation of stimuli and not conduction velocity (162), but no measurements were made on refractory period. For that reason it is impossible to offer an opinion on the relevance of this very stimulating observation. Their further point, that auricular extrasystoles caused by intravenous injection of minute doses of aconitine disappear during vagal stimulation whereas the repetitive discharges which follow topical application of aconitine are accelerated, may be explained by the dual action of vagal stimulation or of cholinergic substances described above, if we suppose that stronger concentrations of aconitine also reduce the refractory period (172). In the latter paper Scherf and Terranova held that the following observations spoke against a circus movement as the underlying mechanism: i. the reappearance of the arrhythmia after cooling; ii. the slow evolution of the reappearing flutter without any change in the form of the P wave after cooling; iii. the production of auricular pauses during the flutter by means of vagal stimulation.

In the opinion of the reviewer none of these arguments alone is absolutely decisive, though together they are formidable. In the first place it seems impossible that only a single point on the surface of the heart should be cooled; the effective cooling must spread over an area surrounding the thermode. We have no information about the limits of this area. Nor indeed have we the necessary basic information about the action of this drug on the fundamental properties of cardiac muscle. Any stimulus, for instance one arising from the normal pacemaker, entering an area recovering from cooling and already damaged by aconitine might restart a small circus movement. The slow evolution of the reappearing flutter might be due to the gradual recovery in conduction velocity and concomitant reduction in refractory period. And finally the observation of auricular pauses might be due either to occasional blocks developing around the periphery of the circuit, or to inconstant changes in the circuit path. In a single isolated auricle showing repetitive discharges, varying degrees of block have often been observed, and sudden changes of discharge frequency have also been seen, which sometimes proved to be due to an alteration of the circuit path (41). These considerations make it desirable to suspend judgment on the question of whether the aconitine discharge really does arise from a unicellular type of ectopic focus. The analogies drawn with other tissues, particularly with nerve, are very powerful, but they do not constitute scientifically decisive arguments.

It is strange that, while so much attention has been concentrated on the effect

of topical applications of aconitine, until recently relatively little work has been done on the very similar action of cholinergic derivatives. In 1940 Nahum and Hoff (83) found that the application of strips of filter paper (0.2 x 1.0 cm.) soaked in acetyl- β -methylcholine 1:500 to 1:2,000 led in some instances to auricular fibrillation without any further procedures. Local application of atropine prevented this action of the drug. Hirschfelder and Tamcales (79) used this method for testing the ability of some local anaesthetics to stop auricular fibrillation, and Gertler and Karp (69) used it to test atabrine. If it be objected that sufficient of the drug were thereby absorbed or spread over the surface of the auricle, surely the same argument can be applied to experiments using aconitine? And one is tempted to enquire whether the action of the two substances is fundamentally the same. That appears at first sight the inference to be drawn from Scherf and Chick's (166) more recent study of the arrhythmias caused by topical application of acetylcholine to the auricles or ventricles; they concluded that, as with aconitine, their results could not be explained on the basis of circus movements alone. In a later paper (167) they came to somewhat similar conclusions after experiments with topical applications of veratrine. It is a notable feature of all these experiments that strong concentrations of the substance used are required, whether it be aconitine, acetylcholine or veratrine. From the available literature it is not certain whether this will cause a local area of depolarization. This is a possibility, and, when one considers the very strong shocks required to produce ventricular fibrillation by electrical means, suggests that the explanation for all these phenomena may be of a similar nature. The production of an area of myocardial damage, greater in the centre than at the periphery, and of very roughly circular shape is necessarily a common feature of these methods of producing arrhythmias.

Finally there are the experiments of Prinzmetal *et al.* (147, 148) using high-speed cinematography as well as other methods, on flutter and fibrillation induced in the dog by aconitine and electrical stimulation. Observations have also been reported on the naturally-occurring disease in man. The authors have come to the conclusion that the same basic mechanism, a single ectopic focus, is responsible for auricular premature systoles, paroxysmal tachycardia, flutter and fibrillation. The evidence is hard to assess, particularly in respect of the high-speed films. The movement of the wall of the auricle is of a rather gross character, contraction in one part is accompanied by movement in another, and this causes some difficulty in interpretation. While these experiments provide evidence that under the particular conditions used there is no circus movement of a gross character, the intimate nature of the ectopic focus is still undetermined. And we have to set against this general conclusion that large circus movements were not observed the more recent observations of Rosenblueth and Garcia Ramos (155) of just such circus movements in dogs, as well as the very careful and now classical experiments of Sir Thomas Lewis and his colleagues. It is not possible to come to a final conclusion on the present evidence.

It is indeed difficult to believe that evidence of a *quite unequivocal nature*, on the question of whether a unicellular ectopic focus will discharge repetitively in

the manner suggested, will ever be obtained by the application of drugs to the intact, or even perhaps the isolated auricle alone. The spread of the drug following its application by the methods used hitherto is quite beyond the control of the experimenter, who must then rely upon his recording apparatus to determine the size and nature of the centre emitting the consequent rhythmic discharges. Only when the drug is limited to a single cell, perhaps by injection within that cell, or when records are obtained from a single cell, which alone is discharging repetitively, uninfluenced by its neighbours, will a sound experimental foundation be laid for this hypothesis. It should also be acknowledged that the two mechanisms envisaged as determining the rhythm of an ectopic focus, unicellular or small circus movement, are not mutually exclusive. Both may exist and due account must be taken of this possibility.

THE ACTION OF SUBSTANCES WHICH PREVENT OR STOP ARRHYTHMIAS

Prevention or arrest of experimental or human cardiac arrhythmias has been reported with a depressingly wide variety of chemical substances. But before considering the implications of these findings, it is pertinent to enquire what properties they might be expected to possess. This may be considered either from the point of view of a circus movement, or of a unicellular ectopic focus. As to the former it has been recognized ever since the work of Mines (128) and Garrey (67) that prolongation of the refractory period might reasonably be expected to arrest circus movement. But, as Sir Thomas Lewis pointed out, a simultaneous decrease in the conduction velocity will act in the opposite direction. Indeed, since quinidine was found not only to prolong the refractory period but also to slow conduction, these two actions would well explain both the invariable slowing of the auricle which follows the use of quinidine in flutter or fibrillation and the frequent failure to restore a normal rhythm (112). The practical problem of stopping a circus movement is possibly more complicated still, since as the refractory period is prolonged a time will be reached when the excitatory state is travelling in the relative refractory period of the preceding cycle. In such circumstances conduction velocity may be reduced still further. It follows from what has been said of the hitherto widely accepted theory of the action of quinidine in auricular arrhythmias that to be effective a therapeutic agent should prolong the refractory period without unduly reducing conduction velocity. Here we arrive at a virtual impasse. It is certainly possible to measure accurately under standardized conditions the action of drugs, or of alterations in the environment on both refractory period and conduction velocity in heart muscle. But unfortunately no systematic study on both variables has yet been conducted, in spite of the very strong theoretical reasons for supposing that measurements of either alone will not give a reliable indication of the behaviour of a substance on circus movements. There is a further defect in our knowledge of the basic facts about this subject. It is clear that, provided a drug effects a very considerable prolongation in refractory period, some reduction in conduction velocity may be tolerated, as in quinidine: but we know virtually nothing of the quantitative aspect of this relationship. Finally, there is a case for believing that the

ability of quinidine, in sufficient concentration, to reduce excitability, also may play a part in its therapeutic effect on a circus movement. For if we consider the means by which the excitatory state is propagated from one cardiac muscle fibre to the next, then how does the syncytial bridge between the cells operate in practice? It is almost certain that, as in nerve, the size of the action potential, or of the current produced by the activity of one unit will determine whether the next unit is excited. Thus an over-all reduction in excitability may be the crucial factor in determining that the passage of a circus wave, travelling already within the relative refractory period of the preceding cycle, shall fail to excite the next element in the ring, and hence die out.

A similar analysis may be made of the factors likely to influence an arrhythmia controlled by a unicellular ectopic focus, discharging at a frequency inversely related to the refractory period, of the type considered above. In this instance it is obvious that a prolongation of refractory period will reduce the frequency of discharge, and that a reduction of excitability may cause the discharge to cease. Alterations in conduction velocity would here be irrelevant.

On both theories then, a reduction of excitability, accompanied by a prolongation of refractory period, is desirable on theoretical grounds. If the disturbance is due to a circus movement, due account will also have to be taken of the conduction velocity. As DiPalma and Schultz (48) have pointed out, the ideal "anti-fibrillatory" drug should also have other properties, more in the nature of omitting unpleasant potentialities than in possessing desirable ones. For instance it should not reduce the contractility of cardiac muscle, it should not itself lead to cardiac irregularities, it should not cause unpleasant side-reactions, it should be readily assimilable, and its blood concentration should be easily maintained for an effective period of time.

Perhaps the most extraordinary feature of the substances which have been used in the treatment of cardiac arrhythmias is that quite a number of them appear on occasion to have precipitated yet more serious or even fatal arrhythmias. That this is true of quinidine is widely known, and DiPalma and Schultz (48) have pointed out once again the particular hazard of using quinidine in the presence of myocardial damage or a conduction defect. Perhaps it is unwise to argue from experience in man, however alarming it may be, since in such instances myocardial damage is almost certainly present and it is by no means certain to what extent this may have contributed to the outcome. But what are we to make of the observation that α -fagarine, a drug which has been submitted to extensive experimental and clinical trial as a substitute for quinidine, may also produce ventricular tachycardia and fibrillation on intravenous administration to presumably normal animals (48)? It would be valuable to be assured that this action of α -fagarine is not simply a terminal event brought about by a severe cardiovascular disturbance, and that it really is due to direct myocardial action. There is good evidence that α -fagarine prolongs the refractory period, reduces excitability and slows conduction velocity (10, 43, 132-134). Scherf (165) found that it stopped auricular fibrillation produced in dogs by local application of aconitine, and Tacchini (189) found a similar therapeutic effect in man. Yet

though it may be used successfully clinically, multifocal ventricular extrasystoles may also result, even though a normal sinus rhythm has been restored (171). Recently DiPalma and his colleagues (46, 49) have had a very similar experience with another compound, a synthetic analogue of α -fagarine. On the other hand, there appear to be no records of similar accidents with substances like procaine, possibly because its toxic effect on systems other than the cardiovascular are of predominant importance.

While there is not sufficient evidence to suggest how these substances themselves cause ventricular tachycardia or fibrillation, one point may be made. In considering the effects of various forms of damage to the myocardium, in particular that due to ether and chloroform, it was observed that deep chloroform (111, 192) and ether anaesthesia (73) reduced the liability to arrhythmias. It is therefore unlikely that all types of severe myocardial damage by drug action predispose to ventricular fibrillation.

Consideration of the other properties shown by substances which have been used successfully in the treatment of experimental or clinical arrhythmias does not throw much further light on the nature of these arrhythmias. They all prolong the refractory period, and reduce excitability and conduction velocity, in so far as these have been measured by reliable methods. It is also interesting that these substances are local anaesthetics (37, 38, 48) (data on this point appear to be lacking for α -fagarine), and so may be presumed to have similar actions upon nerve. Here is a further analogy, similar to that drawn in preceding sections of this review on the ability of the same substances to elicit repetitive discharges both in nerve and cardiac muscle. It is difficult to disregard the implication that the fundamental mechanism which determines excitability is the same in both tissues, even though the time course of the action potential shows such differences.

METHODS

There are only a limited number of methods for investigating the quinidine-like action of drugs, on which sufficient work has been done to assess their reliability. Whether even these are likely to pick out the right therapeutic compound is still a matter of opinion. There is no doubt that the basis of all these methods is empirical, for there are but two alternatives. Either you select a method of producing a cardiac arrhythmia experimentally and see if one substance is better than another at preventing or arresting it, or you measure the change caused by the drug in some property of cardiac muscle which on theoretical grounds is thought to be of crucial importance in the development or maintenance of that arrhythmia. The assumption involved in the latter method is far from being a matter of scientific certainty.

1. *Levy's method*

By this is meant all those methods in which chloroform, benzol or cyclopropane are used in combination with adrenaline to produce ventricular arrhythmias. This method has already been discussed in considerable detail above, and

reference has been made to the standardization of technique introduced by Meek, Hathaway and Orth (126), and to the necessity of considering the role played by the blood pressure in this procedure. (There is evidence that quinine (96) and quinidine (39) may reduce the pressor action of adrenaline while small doses of procaine (117), may actually increase it.) Wégria and Nickerson (203) have also introduced a modification of the method, in which the use of benzol is preferred as being more reliable in the production of arrhythmias, and in which ventricular fibrillation is stopped by "A.C. counter-shock." A number of trials are therefore possible before and after the administration of the substance to be tested. This method obviously has much to recommend it, as adequate control observations are possible on the same animal, and it is not necessary to rely so much on statistical treatment of the fate of groups of animals.

There is one further critical consideration which must be borne in mind, that is the effect which a compound of potential therapeutic use may have in decreasing or increasing the release of endogenous sympathomimetic substances, or in modifying directly the cardiac action of adrenaline or of the anaesthetic used. Perhaps an example of this potential fallacy may be given. It is conceivable that a substance may prevent adrenaline having its myocardial action by competing for the same receptors in the heart; such a substance would be expected to prevent adrenaline-cyclopropane induced arrhythmias, but would not necessarily prevent or cure the human disease. According to Stutzman and Allen (186), as mentioned above, prolonged exposure to cyclopropane itself has an "adrenolytic" action. It is interesting that recently Kraye and his collaborators (97-99, 102-104) have found a number of substances that antagonized the cardio-accelerator action of adrenaline; quinidine also possessed the same property (100). It is highly unlikely that this is a specific action of quinidine in the sense used above; it is probably one manifestation of the direct myocardial action of quinidine, yet the precise implications of this antagonism are intriguing. Another example of the possible difficulties in interpretation using this method is to be found in experiments with local anaesthetics like procaine. Since all methods suggest that procaine prevents arrhythmias, and its use in surgical practice is satisfactory, there seems little doubt of its efficacy. This does not mean to say that successful experiments with the cyclopropane-adrenaline method alone have established its efficacy *with scientific accuracy*, for procaine is a sufficiently potent local anaesthetic to block both afferent and efferent nervous pathways (27, 117, 182). One group of workers at least have come to the conclusion that even procaine affords no practical protection (89), though the methods they used differed in some details from those of other workers (31, 187). It appears that the effect of procaine in such tests is very shortlived, as is only to be expected by its rapid breakdown and removal from the bloodstream (28, 115, 178, 180), and this may well explain the discrepancy. It is a little surprising that since this substance (like many others) is administered by slow intravenous infusion during anaesthesia pharmacologists have not made wider use of this method of administration. No doubt this will be necessary in the future. The multitude of variables involved also explains why there are discrepancies in the literature as to the efficacy of

ergotamine (8, 143, 145) and of procaine amide (119, 135). Nickerson and Smith (143) have even remarked on a seasonal variation in sensitivity to cyclopropane-adrenaline induced arrhythmias.

Much useful information has been gained with this method, but it is quite clearly incapable of giving a quantitative answer, and it is even doubtful whether substances possessing a small degree of activity can be distinguished from those possessing none at all. There does not seem to be any fundamental reason for using cyclopropane rather than chloroform or benzol, though there is perhaps the practical reason that more recent (American) work has been carried out with the former, and so the results from different laboratories may be more readily compared.

2. *Barium and aconitine methods*

These include methods based either on the intravenous injection or the topical administration of barium chloride, aconitine, acetylcholine, acetyl- β -methylcholine and other substances. It seems likely that more such compounds will come to hand in the future, as in the recent report of ventricular arrhythmias caused by 2489F (93) and of auricular fibrillation caused by some propylene glycol derivatives (181). All such methods are bound to be of qualitative value only, and some of the considerations discussed in relation to the "Levy methods" also apply, since these experiments are usually performed in whole animals, and not all the variables have yet been investigated. Mention should also be made of the use by Tripod (195) of perfusion of the Langendorff (106) preparation of the cat or rabbit heart with varying concentrations of aconitine to produce arrhythmias. In conjunction with some other quantitative test, these methods give greater assurance before proceeding to clinical trial, and they appear to have been used in this way occasionally (25).

3. *Arrhythmias produced in the whole animal by electrical stimulation*

This includes the oldest experimental method of producing auricular or ventricular arrhythmias, by direct faradization. Wiggers and Wégria (210) in a very lucid and critical exposition of these methods came to the conclusion that the common feature of them all was the interposition of a *single* effective shock during the vulnerable period. Rhythmic stimulation by an induction coil, or by more reliable methods such as rectangular pulses of predetermined duration or sine-wave pulses, was therefore a more elaborate and less satisfactory procedure. Evidence was adduced to show that prolonged oscillating currents give erratic responses (204, 205) and that the duration of fibrillation thus excited in cats, or the number of times spontaneous recovery occurs (58), are not reliable indications of a therapeutic action. On these grounds they based their conclusion that the "fibrillation threshold" in dogs as measured by a single shock delivered during the vulnerable period was to be preferred. However, Wégria and Nickerson (203) later came to the conclusion that this method was rather difficult and tedious. The current strength required was very large, and it is interesting to observe that in a recent paper DiPalma, Lambert, Reiss and Schultz (46, 49),

reverting to the use of rhythmic pulses (wave-form and duration not recorded) at a fixed frequency of 600 per minute also had to use very strong stimuli (threshold between 2 and 8 ma.) in order to produce ventricular fibrillation in cats. For comparison it is worth recording the fact that the threshold current required to excite the isolated mammalian auricle is less than 0.1 ma. (rectangular pulse form, duration 2-5 m.sec.), and that repetitive discharges can be initiated by an interpolated pulse very little stronger, usually not more than 0.2 to 0.3 ma., and sometimes even less. DiPalma and his colleagues (46) found their method satisfactory for quantitative evaluation of the relative activity of a number of synthetic compounds, though they do not appear to have checked it by using substances whose activity has been tested by other methods, and the criticisms offered by Wiggers and Wégria (210) still apply. Only the latter appear to have had practical experience of both types of method, though admittedly they did not use cats. It may well be that the use of cats, in which the fibrillation is evanescent, is better than that of dogs, in which ventricular fibrillation is usually fatal, and has to be stopped by strong electrical stimulation ("serial defibrillation" or A.C. countershock).

Whether the form of electrical stimulation is by a single pulse delivered during the vulnerable period, or by rhythmic pulses from an induction coil or less variable form of stimulator, and whatever the view held as to the mechanism whereby the arrhythmia is thus started, there can surely be no doubt as to one general conclusion. If a substance reduces the excitability of the myocardium a greater current strength will have to be used either to excite a single response or to cause flutter or fibrillation. In that case the ability of a substance to raise the "fibrillation threshold" the expression used by Wiggers and Wégria, is not necessarily an indication of a specific ability to prevent arrhythmias; it may be due to reduced myocardial excitability. Of course it is very likely that reduced myocardial excitability is one of the fundamental properties required in an effective therapeutic agent for treating cardiac arrhythmias, but there are certainly more direct, more reliable and less drastic methods of measuring excitability than by measuring the "fibrillation threshold" in any of the ways described. Wiggers and Wégria have considered this proposition, and took the view that it still remains to be demonstrated that there is a relationship between the strength of the diastolic stimulus necessary to evoke a premature contraction and the stimulus necessary to cause fibrillation. This is indeed the essence of the problem, but in view of the very well established fact that quinidine and procaine (to take two specific examples), both substances which raise the fibrillation threshold (202, 210), also reduce myocardial excitability (17, 51, 121), it is really up to those who use these methods to show that they are measuring the specific ability of a substance to prevent fibrillation rather than reduce myocardial excitability. Indeed, in the summary to their paper, Wiggers and Wégria (210) also state their belief that the "fibrillation threshold" of the dog's ventricle as measured by their method "takes into account the irritability of non-refractory myocardial fractions during the vulnerable phase of systole, and also any local state that may be necessary for the initiation of ventricular

fibrillation." To the reviewer this statement seems to be a virtual abandonment of the whole position, for if the method takes into account myocardial irritability or excitability, what grounds have we for supposing that any active substance affects the local state necessary for the initiation of fibrillation? Until it can be conclusively demonstrated that these methods do more than measure (somewhat indirectly) the effect of a substance on myocardial excitability, it is illogical to attach to this measurement a greater significance.

4. *The ability to stop an established arrhythmia*

The ability of a substance to stop an established arrhythmia in an experimental animal is clearly an excellent demonstration of therapeutic action before proceeding to clinical trial. By its very nature however it is not a test that can conveniently be put on a quantitative basis. There are isolated examples in the literature of substances which are claimed to have been effective in arresting ventricular fibrillation (17), but some doubt is attached to this interpretation because of the cardiac massage and other procedures which are necessary at the same time. Of more practical interest is the development by Rosenblueth and García Ramos (155) of a method, already referred to above, of ensuring that auricular flutter once established by electrical stimulation in the dog shall continue without spontaneous arrest. Brown and Acheson (27) have demonstrated the ability of procaine and two related compounds to cause reversion to a sinus rhythm in this preparation. Auricular fibrillation induced by the administration of drugs could presumably be used in the same fashion, if it were certain that spontaneous reversion did not occur during the course of the experimental period.

5. *Isolated preparations*

Isolated preparations of the auricle or ventricle have been used very extensively for the investigation of the fundamental properties of cardiac muscle. The papillary muscle of the cat's heart has the particular advantage of being readily isolated and containing mainly parallel bundles of fibres (33, 72). Auricular muscle preparations are more complex in nature. One of the latter was used for the determination of the "maximal rate" at which the muscle would follow electrical stimuli, as a readily determined index of the refractory period (37, 38). This method has been used by a number of workers to determine quinidine-like activity (1, 10, 45, 53, 55, 59, 122) and the observation that the percentage decrease in maximal rate is linearly related to the logarithm of the drug concentration has been confirmed. However a number of radical modifications in the procedure adopted is desirable. It should be explained that this method was developed towards the end of World War II because of the sudden scarcity of quinidine. Time could not then be spent in constructing the apparatus for direct measurement of the refractory period, and an approximation which was easy to measure (but less easy to interpret) was adopted instead. Now that the urgency no longer exists the method should be superseded by one in which the refractory period is measured directly, and not merely the refractory period but excitability and conduction velocity at the same time. The isolated guinea-pig or rabbit

auricle can be used at 37°C for this purpose, with action potentials recorded from one or more points as an index of activity. Apart from other considerations, the slope of the curve relating refractory period and temperature is much less steep at 37°C. The auricle is driven by a just supra-threshold stimulus at a frequency just greater than its natural sinus rhythm, and a second stimulus interpolated to test the return of excitability. It has been found desirable to pay attention to the following points (40, 41); the refractory period must be measured by plotting the whole of the excitability curve, so that the limits of both absolute and relative refractory period are delineated; the refractory period so measured varies with the frequency of the driving stimulus (as is well-known), and with the relative frequency at which the test stimulus is applied and with the strength of the driving stimulus. A little consideration of these observations shows that it is most unlikely that the "maximal rate" as previously determined is simply related to refractory period as usually defined. And it is doubtful whether many of the figures recorded for refractory period in the literature are quantitatively reliable, since the influence of all these variables has not always been recognized. It may be argued that the "maximal rate" is a useful approximation, and that in practice it appears to give an index of activity which is roughly justified by experience. Nevertheless its future use cannot be regarded as anything but a retrograde step, when the alternative is presented of direct measurement of properties which are defined so as to be as unambiguous as possible and which are generally regarded as fundamental to the nature of cardiac muscle. Similar measurements on comparable preparations of ventricular muscle are also desirable, and DiPalma and Mascarello (47) have evidently made a beginning in this direction. Finally, it is perhaps appropriate to emphasize once again the importance of gaining some information about the action of potential anti-fibrillatory drugs on the membrane potentials of single cardiac muscle fibres.

6. General considerations

The danger of relying too much on results obtained by bioassay on isolated tissues is well recognized; in the intact animal there are so many other variables which play a part. In this particular field concerned with the search for drugs which will arrest or prevent cardiac arrhythmias many, but possibly not all, of these variables have been recognized. It would be most unwise to proceed without tests in the whole animal as well as on isolated tissues, and some combination of these techniques may prove to be the best. On the isolated auricle or ventricle it is practicable to devise a routine method of comparing the quantitative effects of drugs on the fundamental properties of the heart. A selection of those which seem most promising might then be tested on one or more of the qualitative whole animal techniques, of which Wégria and Nickerson's benzol-adrenaline technique with A.C. countershock to stop ventricular fibrillation (203), Scherf's aconitine method (169) or Brown and Acheson's use (27) of Rosenbluth and García Ramos' auricular flutter method have much to commend them.

Finally it must be borne in mind that drugs of this type are required for dif-

ferent therapeutic purposes, for arresting established auricular flutter or fibrillation, for preventing ventricular fibrillation during intra-thoracic operations and for preventing paroxysmal tachycardia. For some of these purposes it is clearly desirable that an effective drug should be assimilated readily by mouth, and that the blood concentration should not fall too rapidly; quinidine fills this particular requirement moderately well. For other purposes, as during surgery, a long duration of action is not so essential, and procaine, which is more rapidly eliminated, may be given by slow infusion intravenously; the blood concentration is in this instance more directly under the control of the anaesthetist. In setting up a systematic investigation of these drugs, therefore, some consideration of the duration of a drug's therapeutic effect is necessary, as well as of its undesirable toxic properties. The fact that the therapeutic action is transient does not preclude it from being very useful in particular clinical applications, and reduces the danger of prolonged excessive dosage.

RESULTS

From what has been said on the critique of the various methods, it will be appreciated that it is far from easy to reach any conclusions, on a firm scientific basis, about the quantitative activities of substances tested. Where an author has measured the activity of a number of substances, using the same method, he is naturally entitled to draw conclusions within the limits of reliability of that method; the applicability of these conclusions to the problem of naturally occurring arrhythmias in man is another matter. All this is so obvious that it is perhaps impertinent to restate it. Strict quantitative comparison of results obtained by one technique with those obtained by a method based on a fundamentally different concept is not logically justifiable. Such comparisons are useful in suggesting the lines which new work shall follow, but they cannot be regarded as providing firm evidence of relative activity. This is of course a principle which is generally acknowledged in any scientific enquiry, but it applies with particular force to this field in which so many methods have been used, and in which the relevance of any method to the therapeutic object rests on insecure foundations.

In only a few papers have the activity of any substantial number of compounds been compared by the same worker in the same laboratory over a short period of time (38, 46, 72, 122, 134) and it is not possible to compare the activities recorded in all these series for the reasons discussed above. Apart from these papers a heterogeneous collection of compounds have been studied by widely differing methods, of a variety which it is hardly possible to classify. A general description of some of the more interesting of these classes of compounds and their therapeutic use has been given in a recent review by DiPalma and Schults (48) to which reference may be made. There are only a few selected topics on which further comment seems desirable.

The appended table gives a list of results obtained by a number of authors with different methods, using quinidine and procaine. The wide variation in what is regarded as an effective dosage does not engender very great confidence

in the quantitative value of these methods considered as a whole. It is interesting to observe that among the lowest effective doses recorded for either substance is that needed to restore a normal rhythm in continuing auricular flutter in the dog (Rosenbluth and García Ramos' technique). And this once again makes one wonder what the other methods are really measuring. It is interesting to calculate what the approximate blood levels in some of these experiments

TABLE 1
Results obtained with quinidine and procaine by various methods

DOSE REQUIRED TO PROTECT AGAINST:	SPECIES	QUINIDINE	PROCAINE	REF.
Cyclopropane-adrenaline arrhythmias	Dog	Slight 5 mg./kg. Clearcut 10-15 mg./kg.	Just significant 10 mg./kg.	(143)
	Dog	15 mg./kg.	12-16 mg./kg.	(8)
	Dog		5 mg./kg.	(30)
	Dog	1-20 mg./kg.*		(31) (88)
Chloroform-adrenaline arrhythmias	Dog		4-5 mg./kg.	(180)
Benzol-adrenaline arrhythmias	Dog		8-10 mg./kg.	(178)
	Dog	5-15 mg./kg.		(203)
Electrically induced auricular arrhythmias	Cat and Rabbit		2.5 mg./kg.	(197)
	Dog		10-20 mg./kg.	(79)
	Rabbit	1 mg./kg.		(134)
Electrically induced ventricular arrhythmias	Dog	2.4 mg./kg.	8-10 mg./kg.	(202)
	Cat	3.5 mg./kg.		(210)
	Rabbit	1 mg./kg.		(173) (134)
Auricular fibrillation induced by acetyl- β -methyl choline	Dog		1-6 mg./kg.	(79)
Dose required to arrest continuing auricular flutter	Dog	1-2 mg./kg.	1-2 mg./kg.	(1) (27)

* All doses effective 10 min. after injection; thereafter duration of protection varied with the size of dose.

may have been. After a rapid intravenous injection of procaine into a dog, in a dose of 10 mg./kg., the blood procaine level rose in 10 seconds to 70 mg./L. and after 5 minutes was only 5-10% of this (3.5-7.0 mg./L.) (115). Similar observations have been made on cats (28). Since different workers have tested the protection given by procaine at different times after its injection it is difficult to draw any firm conclusions about the therapeutic blood level required in these experimental procedures. The comparable data on man are more informative.

In clinical use procaine may be infused at rates of 10-60 mg./min. (190), or given in single intravenous injections of 100 mg. (32), sometimes repeated. Blood procaine levels of less than 1 mg./L (26) up to as much as 8 mg./L (90) have been recorded. The blood concentration of quinidine following single oral doses of 0.6-1.0 Gm. in man has also received much attention recently (42, 78, 95, 114, 201). The concentration of quinidine in the plasma at the time of conversion from auricular fibrillation to a normal sinus rhythm has been recorded as varying from 3.3 to over 20 mg./L, though few patients required more than 10 mg./L (95, 185). A concentration of 10 mg./L of either procaine or quinidine would be expected to cause a considerable decrease in the maximal rate of the isolated auricle when it had reached equilibrium (10, 38, 122), and has also been shown to prevent the arrhythmias caused by aconitine in the perfused heart (195). There is therefore a reasonable measure of agreement as to the effective concentrations.

STRUCTURE AND ACTION

As DiPalma and Schults (48) have pointed out, the ability of atropine to prevent or to arrest arrhythmias has often been considered, and there is evidence to suggest that under particularly favourable conditions it may sometimes be effective. The implications of this conclusion are less easy to analyse. Atropine is often regarded as the type-substance of a particular class of compounds which antagonize the muscarinic actions of acetylcholine, but it has other properties in addition. It is believed to possess a feeble local anaesthetic action and when used in high concentration reduces the "maximal rate" of the isolated auricle (38). This no doubt is a partial explanation of why it is sometimes effective in preventing cardiac arrhythmias. It also abolishes the effect of acetylcholine in reducing the refractory period, which may be another reason for its efficacy. The use of atropine as a type-substance can therefore be misleading. There are good grounds for preferring the quaternary metho-salt of atropine, or some other quaternary ammonium salt of similar structure and activity for this purpose (92) as such substances do not possess local anaesthetic activity. And it is interesting to recall that quaternary salts of local anaesthetics which have a quinidine-like action are themselves inactive. This raises once again the theoretical consideration as to which constituent, the base or cation, of a solution of a local anaesthetic (or indeed atropine) is the active one, and indicates the desirability of repeating Trevan and Boock's (194) experiments, using cardiac muscle instead of the cornea, and measuring excitability and refractory period instead of local anaesthetic activity.

Since quantitative data on the relation between structure and action in this field are so limited it is unprofitable to discuss the details of this aspect of the problem further than has already been done (38, 46); yet there are certain general considerations which may be worth mentioning. It has already been pointed out that saligenin, a moderately powerful local anaesthetic of very different structure than procaine, possesses no basic group and has no action on the auricle in high concentrations (38). But Diethyl-amino-ethanol and some of its

derivatives (27, 118, 154) are active, and it is evident from this work that the aromatic group is not essential for activity, though its presence very greatly increases activity. Whether this is accompanied by a yet greater increase of toxicity may depend on the nature of the aromatic group. Another transformation of the parent molecule has yielded procaine amide (119) which is more stable than procaine in plasma, though less active. It can be taken by mouth and has been used therapeutically in man (18), where it is said to be more effective and less toxic than quinidine for the treatment of ventricular arrhythmias.

Although all these substances which have proved effective in preventing cardiac arrhythmias possess a more or less distant structural relationship to the well-known local anaesthetics, and so far as they have been examined are themselves local anaesthetics, it should not therefore be concluded that "quinidine-like activity" is necessarily confined to this broad structure type. The possibility that dibenamine may have a direct myocardial action as well as an adrenolytic effect has been debated (1, 142). The general anaesthetics certainly possess rather similar attributes. They block conduction in nerve, and there is good reason to suppose that chloroform and ether will if given in sufficient concentration prevent the development of cardiac arrhythmias (73, 111, 192). These general anaesthetics have a very considerable action on the central nervous system, quinidine and procaine a much less considerable effect, and procaine amide and diethyl-amino-ethanol less still according to early reports. There is a reasonable chance of yet further improvement.

In conclusion, it seems that because of our lack of certainty about the basic physiological mechanisms responsible for the various forms of cardiac arrhythmias in man, and because of the unreliability of methods for testing appropriate therapeutic agents in animals, we are bound to proceed for some time to come on an empirical basis. This is occasioned by the multitudinous factors involved in investigations of activity and toxicity in the whole animal. Nevertheless it is felt that systematic experiments on isolated tissues, combined with greater realisation of the factors which may influence the outcome of experiments in the whole animal, are likely to improve the position in the future, and lead to a more rational appreciation of the fundamental properties desired in an effective therapeutic agent.

SUMMARY

1. The methods of producing cardiac arrhythmias in experimental animals have been surveyed from the point of view of the variables which determine whether an arrhythmia results. These include the level of the blood pressure, the oxygen saturation of the blood, myocardial damage and perhaps the endogenous secretion of sympathomimetic amines.

2. Some of the methods for producing cardiac arrhythmias, such as the use of barium chloride, the veratrum alkaloids and aconitine, suggest an analogy with nervous tissue, in which these substances cause repetitive discharges, super-

normality and an increase in the negative after-potential. This is only part of the evidence which suggests that many of the variables which influence the outcome of these and other procedures in the intact animal may have a common basis in their effect on the membrane potentials of individual cardiac muscle fibres.

3. The meaning of the term "ectopic focus" has been examined. Two hypotheses which may explain the origin of discharges from an ectopic focus were considered in relation to the experimental evidence.

4. In the light of the evidence outlined above, the prerequisites for a therapeutic agent to prevent or arrest arrhythmias have been discussed, and the methods used for measuring "quinidine-like" activity criticised. There seems little likelihood of removing these methods from their present empirical foundation without further knowledge of the behaviour of single myocardial cells. In the meantime it is desirable to recognize the tacit assumptions on which these methods are based.

REFERENCES

1. ACHESON, G. H., FARAH, A. AND FRENCH, G. N.: Some effects of dibenzyl β -chloroethylamine (dibenzamine) on the mammalian heart. *J. Pharmacol. & Exper. Therap.*, **97**: 455-465, 1949.
2. ACIERNO, L. J. AND DIPALMA, J. R.: The effects of ether, cyclopropane and chloroform on the isolated auricle of the cat. *Anesthesiology*, **12**: 567-573, 1951.
3. ADRIAN, E. D.: The recovery process of excitable tissues. I. *J. Physiol.*, **54**: 1-31, 1920.
4. ADRIAN, E. D.: The recovery process of excitable tissues. II. *J. Physiol.*, **55**: 193-225, 1921.
5. ALLEN, C. R., HOFFFLICH, E. A., COOPER, B. M., AND SLOCUM, H. C.: Influence of the autonomic nervous system upon spontaneous cardiac arrhythmias during cyclopropane anesthesia. *Anesthesiology*, **6**: 261-267, 1945.
6. ALLEN, C. R., STUTZMAN, J. W., FOREGGER, R. AND MEEK, W. J.: The cardiac arrhythmias which occur spontaneously in cats during cyclopropane anesthesia. *Anesthesiology*, **3**: 530-539, 1942.
7. ALLEN, C. R., STUTZMAN, J. W. AND MEEK, W. J.: The production of ventricular tachycardia by adrenalin in cyclopropane anesthesia. *Anesthesiology*, **1**: 159-166, 1940.
8. ALLEN, C. R., STUTZMAN, J. W., SLOCUM, H. C. AND ORTH, O. S.: Protection from cyclopropane-epinephrine tachycardia by various drugs. *Anesthesiology*, **2**: 503-514, 1941.
9. ALLEN, W. F.: Contributing factors to the pulse changes resulting from injection of epinephrin in rabbits. *J. Pharmacol. & Exper. Therap.*, **50**: 70-78, 1934.
10. ALLEN, G. A. AND ELLIS, C. H.: Comparative actions of certain compounds like alpha-fagarine. *J. Pharmacol. & Exper. Therap.*, **94**: 416-425, 1948.
11. AMBRACHE, N.: The nicotine action of substances supposed to be purely smooth-muscle stimulating. *J. Physiol.*, **110**: 164-172, 1949.
12. ANDRUS, E. C. AND CARTER, E. P.: The refractory period of the normally-beating dog's auricle; with a note on the occurrence of fibrillation following a single stimulus. *J. Exper. Med.*, **51**: 357-367, 1930.
13. BARDIER, E. AND STILLMUNKER, A.: La syncope adrénalino-chloroformique. *Arch. internat. de pharmacodyn. et de therap.*, **27**: 375-414, 1923.
14. BARMAN, J. M.: El etileno y el ciclopropano no hacen segregar adrenalina. *Rev. Soc. argent. de biol.*, **15**: 490-491, 1939.
15. BATRO, A. AND LANARI, A.: Injection intra-carotidienne d'acétylcholine chez l'homme. *Compt. Rend. Soc. biol.*, **125**: 541-542, 1937.
16. BEATTIE, J., BROW, G. R. AND LONG, C. N. H.: Physiological and anatomical evidence for the existence of nerve tracts connecting the hypothalamus with spinal sympathetic centres. *Proc. Roy. Soc. B.*, **106**: 253-275, 1930.
17. BECK, C. S. AND MAUTZ, F. R.: The control of the heart beat by the surgeon. *Ann. Surg.*, **106**: 525-537, 1937.
18. BERRY, K., GARLETT, E. L., BELLETT, S. AND GEFTER, W. I.: Use of pronestyl in the treatment of ectopic rhythms. *Am. J. Med.*, **11**: 431-441, 1951.
19. BHATIA, B. B. AND BURN, J. H.: The action of ether on the sympathetic system. *J. Physiol.*, **78**: 257-270, 1933.
20. BILAMA, V. G. AND VAN DONGEN, K.: Experimentelle Therapie des Flatterns und Flimmerns der Herzens. *Ergebn. Physiol.*, **41**: 1-25, 1939.
21. BLUMENTHAL, B. AND OPPENHEIMER, E. T.: Method for the study of ventricular fibrillation. *Am. Heart J.*, **18**: 363-367, 1939.
22. BOEHM, R.: Heffer's handbuch der experimentellen Pharmacologie. II. **1**: 283-303, 1920.
23. DE BOER, S.: On the fibrillation of the heart. *J. Physiol.*, **54**: 400-414, 1921.
24. BOUCKAERT, J. J. AND HEYMANS, C.: Syncope adrénalino-chloroformique et sinus carotidiens. *Compt. Rend. Soc. Biol.*, **105**: 878-880, 1930.

25. BOVET, D., FOURNEAU, E., TRÉPOUËL, J. and STRICKLER, H.: Le diéthylaminoéthoxy-2-diphényl (1262F). Propriétés pharmacologiques et activité sur la fibrillation du cœur. *Arch. internat. de pharmacodyn. et de thérap.*, **62**: 234-260, 1939.
26. BRODIE, B. B., LIEP, P. A. and PORT, R.: The fate of procaine in man following its intravenous administration and methods for the estimation of procaine and diethylaminoethanol. *J. Pharmacol. & Exper. Therap.*, **94**: 359-366, 1948.
27. BROWN, B. B. and ACHESON, G. H.: The influence of procaine and some related compounds upon experimental auricular flutter in the dog. *J. Pharmacol. & Exper. Therap.*, **102**: 200-207, 1951.
28. BURGEN, A. S. V. and KEELE, C. A.: Quantitative studies of procaine metabolism in the cat. *Brit. J. Pharmacol.*, **3**: 128-136, 1948.
29. BURN, J. H.: The relation of nerve supply and blood flow to sweating produced by pilocarpine. *J. Physiol.*, **56**: 232-247, 1922.
30. BURSTEIN, C. L. and MARANGONI, B. A.: Protecting action of procaine against ventricular fibrillation induced by epinephrine during cyclopropane anesthesia. *Proc. Soc. Exper. Biol. & Med.*, **43**: 210-212, 1940.
31. BURSTEIN, C. L., MARANGONI, B. A., DE GRAFF, A. C. and ROVENSTINE, E. A.: Laboratory studies on the prophylaxis and treatment of ventricular fibrillation induced by epinephrine during cyclopropane anesthesia. *Anesthesiology*, **1**: 167-186, 1940.
32. BURSTEIN, C. L., WOLOSHIN, G. and NEWMAN, W.: Electrocardiographic studies during endotracheal intubation. II. Effects during general anesthesia and intravenous procaine. *Anesthesiology*, **11**: 299-312, 1950.
33. CATTALL, M. K. and GOLD, H.: The influence of digitalis glucosides on the force of contraction of mammalian cardiac muscle. *J. Pharmacol. & Exper. Therap.*, **62**: 116-125, 1938.
34. CHARLIER, R. and KLUTZ, A.: Tachycardie ventriculaire expérimentale. III. Action protectrice de deux amines sympathicomimétiques. *Arch. internat. de Pharmacodyn. et de thérap.*, **87**: 269-274, 1951.
35. CHENOWETH, M. B.: Ventricular fibrillation induced by hydrocarbons and epinephrine. *J. Indust. Hyg. & Toxicol.*, **20**: 151-158, 1946.
36. DAMESHEK, W., LOMAN, J. and MYERSON, A.: The effect on the normal cardiovascular system of acetyl-beta-methylcholine chloride, atropine, prostigmin, benzedrine—with especial reference to the electrocardiogram. *Am. J. M. Sc.*, **195**: 88-103, 1938.
37. DAWES, G. S.: Synthetic substitutes for quinidine. *Brit. M. J.*, **1**: 43-45, 1946.
38. DAWES, G. S.: Synthetic substitutes for quinidine. *Brit. J. Pharmacol.*, **1**: 90-112, 1946.
39. DAWES, G. S.: Unpublished observations.
40. DAWES, G. S. and VANE, J. R.: Repetitive discharges from the isolated atria. *J. Physiol.*, **112**: 28F, 1951.
41. DAWES, G. S. and VANE, J. R.: Unpublished observations.
42. DELEVETT, A. F. and POINDEXTER, C. A.: Plasma concentrations of quinidine with particular reference to therapeutically effective levels in two cases of paroxysmal nodal tachycardia. *Am. Heart J.*, **32**: 697-703, 1946.
43. DEULOFOEN, V., LABRIOLA, R., ORIÁ, O., MORSENET DE ESPANÉS E. and TAQUINI, F.: Fagarine, a possible substitute for quinidine. *Science*, **102**: 69-70, 1945.
44. DEUTSCH, A. and LUNDIN, G.: Effects of minute amounts of barium on cardiac muscle. *Acta physiol. Scandnav.*, **11**: 373-379, 1946.
45. DEWE, P. B. and GRAHAM, J. D. P.: The antihistamine substance 2786 RP. *Brit. J. Pharmacol.*, **1**: 278-286, 1946.
46. DIPALMA, J. R., LAMBERT, J. L., REISS, R. A. and SCHULTZ, J. E.: Relationship of chemical structure to anti-fibrillatory potency of certain alpha fagarine like compounds. *J. Pharmacol. & Exper. Therap.*, **90**: 251-257, 1950.
47. DIPALMA, J. R. and MASCATELLO, A. V.: Analysis of the actions of acetylcholine, atropine, epinephrine and quinidine on heart muscle of the cat. *J. Pharmacol. & Exper. Therap.*, **101**: 243-248, 1951.
48. DIPALMA, J. R. and SCHULTZ, J. E.: Antifibrillatory drugs. *Medicine*, **29**: 123-168, 1950.
49. DIPALMA, J. R., SCHULTZ, J. E., REISS, R. A. and LAMBERT, J. L.: Pharmacological study and clinical use of an alpha fagarine like compound (N-methyl-N-(3,4 dimethoxybenzyl)-2-(4 methoxy phenyl)-ethylamine-HCl). *J. Pharmacol. & Exper. Therap.*, **90**: 258-267, 1950.
50. DRAPER, M. H. and WEIDMANN, S.: Cardiac resting and action potentials recorded with an intracellular electrode. *J. Physiol.*, **115**: 74-94, 1951.
51. DRURY, A. N., HORSFALL, W. N. and MUNLY, W. C.: Observations relating to the action of quinidine on the dog's heart; the refractory period of, and conduction in, ventricular muscle. *Heart*, **9**: 365-374, 1922.
52. DUN, F. T. and FENG, T. P.: Studies on the neuromuscular junction XX. The site of origin of the junctional after-discharge in muscles treated with quinine, barium or eserine. *Chinese J. Physiol.*, **13**: 433-444, 1940.
53. DUTTA, N. K.: Some pharmacological properties common to antihistamine compounds. *Brit. J. Pharmacol.*, **4**: 281-289, 1949.
54. ECLER, J. C. and HOFF, H. E.: The rhythm of the heart beat. *Proc. Roy. Soc. B.*, **115**: 307-368, 1934.
55. DE ELIÖ, F. J.: The action of acetyl choline, adrenaline and other substances on the refractory period of the rabbit auricle. *Brit. J. Pharmacol.*, **2**: 131-142, 1947.
56. ELLIOTT, T. R.: The control of the suprarenal glands by the splanchnic nerves. *J. Physiol.*, **44**: 374-409, 1912.
57. ERLANGER, J. and GASSER, H. S.: Electrical signs of nervous activity. University of Pennsylvania Press, Philadelphia, 1937.
58. ETTINGER, G. H.: The reaction of the cat to electrical currents directed through the heart. *Am. J. Physiol.*, **111**: 406-415, 1935.

59. FARAH, A. AND MOOK, W.: The action of mercurial diuretics on the effective refractory period, electrical excitability and conduction velocity in the mammalian heart. *J. Pharmacol. & Exper. Therap.*, **102**: 125-131, 1951.
60. FASTIER, F. N.: Electrocardiographic features of "adrenaline syncope." *J. Physiol.*, **112**: 359-366, 1951.
61. FASTIER, F. N. AND SMIRK, F. H.: Some properties of amarin, with special reference to its use in conjunction with adrenaline for the production of idio-ventricular rhythms. *J. Physiol.*, **107**: 318-331, 1948.
62. FAWAZ, G.: The mechanism by which N,N-dibenzyl-chloroethylamine protects animals against cardiac arrhythmias induced by sympathomimetic amines in presence of cyclopropane or chloroform. *Brit. J. Pharmacol.*, **6**: 492-498, 1951.
63. FENG, T. P.: Studies on the neuromuscular junction VII. The eserine-like effects of barium on motor nerve-endings. *Chinese J. Physiol.*, **12**: 177-196, 1937.
64. FISHER, C. W., BENNETT, L. L. AND ALLARWALA, A.: The effect of inhalation anesthetic agents on the myocardium of the dog. *Anesthesiology*, **12**: 19-26, 1951.
65. GARR, S. AND CHENOWETH, M. B.: Studies on hydrocarbon-epinephrine induced ventricular fibrillation. *J. Pharmacol. & Exper. Therap.*, **94**: 12-18, 1948.
66. GARCIA RAMOS, J.: Estudios sobre el flutter y la fibrilación. VIII. La anisodromia en el musculo cardiaco y su relacion con las extrasistolas y la fibrilación. *Arch. Inst. Cardiol. Mexico*, **19**: 39-54, 1949.
67. GARREY, W. E.: The nature of fibrillary contraction of the heart. Its relation to tissue mass and form. *Am. J. Physiol.*, **33**: 397-414, 1914.
68. GARREY, W. E.: Auricular fibrillation. *Physiol. Rev.*, **4**: 215-250, 1924.
69. GERTLER, M. M. AND KARP, D.: Effect of atabrine on auricular fibrillation in the dog. *Proc. Soc. Exper. Biol. & Med.*, **64**: 213-218, 1947.
70. GILSON, A. S.: Agents causing cardiac supernormality. *Proc. Soc. Exper. Biol. & Med.*, **41**: 1-2, 1939.
71. GRAHAM, H. T. AND GASSER, H. S.: Modification of nerve response by veratrine, protoveratrine and aconitine. *J. Pharmacol. & Exper. Therap.*, **43**: 163-185, 1931.
72. GRINER, T. H. AND GARR, S.: The influence of drugs on the irritability and automaticity of heart muscle. *J. Pharmacol. & Exper. Therap.*, **98**: 215-223, 1950.
73. GREISHEIMER, E. M., ELLIS, D., OPPENHEIMER, M. J. AND ROBINSON, H. W.: Use of ether in prevention of cyclopropane-epinephrine-induced arrhythmias. *Federation Proc.*, **10**: 55, 1951.
74. GUNN, J. A. AND MARTIN, P. A.: Intrapericardial medication and massage in the treatment of arrest of the heart. *J. Pharmacol. & Exper. Therap.*, **7**: 31-55, 1915.
75. HARRIS, A. S. AND MOE, G. K.: Idioventricular rhythms and fibrillation induced at the anode or the cathode by direct currents of long duration. *Am. J. Physiol.*, **136**: 318-331, 1942.
76. HAZARD, R. AND QUINQUAND, A.: L'yohimbine, paralysant de baryum vaso-constricteur. *Compt. rend. Soc. biol.*, **139**: 601-602, 1945.
77. HEYMANS, C., BOUCKAERT, J. J. AND SAMAN, A.: Influences des variations de la teneur du sang en oxygène et en CO₂ sur l'excitabilité réflexe et directe des éléments centraux et périphériques des nerfs cardio-régulateurs. *Arch. internat. de pharmacodyn. et de therap.*, **48**: 457-487, 1934.
78. HIATT, E. P.: Plasma concentrations following the oral administration of single doses of the principal alkaloids of cinchona bark. *J. Pharmacol. & Exper. Therap.*, **81**: 160-163, 1944.
79. HIRSCHFELDER, A. D. AND TAMCALES, G.: Inhibition of experimental auricular fibrillation by procaine and other substances. *Proc. Soc. Exper. Biol. & Med.*, **50**: 272-274, 1942.
80. HOFF, H. E. AND NAHUM, L. H.: The rôle of adrenaline in the production of ventricular rhythms and their suppression by acetyl- β -methylcholine chloride. *J. Pharmacol. & Exper. Therap.*, **52**: 235-245, 1934.
81. HOFF, H. E. AND NAHUM, L. H.: The nature of ventricular fibrillation following electric shock and its prevention by acetyl- β -methyl choline chloride. *Am. J. Physiol.*, **110**: 675-680, 1935.
82. HOFF, H. E. AND NAHUM, L. H.: The supernormal period in the mammalian ventricle. *Am. J. Physiol.*, **124**: 591-595, 1938.
83. HOFF, H. E. AND NAHUM, L. H.: Production of auricular fibrillation by application of acetyl- β -methylcholine chloride to localized regions on the auricular surface. *Am. J. Physiol.*, **129**: 428, 1940.
84. HOFFMANN, F., HOFFMANN, E. J., MIDDLETON, S. AND TALENNIK, J.: The stimulating effect of acetylcholine on the mammalian heart and the liberation of an epinephrine-like substance by the isolated heart. *Am. J. Physiol.*, **144**: 189-198, 1945.
85. HORLICK, L. AND SENTSIN, A.: The role of anemia in the experimental production of heart block and auricular fibrillation in the dog. *Am. Heart J.*, **38**: 716-731, 1949.
86. V. HUEBER, E. F.: Wirkungsänderungen einiger herzwirksamer Mittel durch Aconitin. *Arch. exper. Path. u. Pharmacol.*, **187**: 541-552, 1937.
87. V. HUEBER, E. F. AND LEHR, D.: Wirkung von Magnesium auf der Vergiftung mit Aconitin. *Arch. exper. Path. u. Pharmacol.*, **189**: 25-44, 1938.
88. HUGGINS, R. A., MORSE, R. A., CHAPMAN, D. W. AND SCHUMACHER, L. F.: The effect of quinidine on arrhythmias induced by cyclopropane and cyclopropane-epinephrine. *Anesthesiology*, **11**: 623-634, 1950.
89. HUGGINS, R. A., MORSE, R. A., HANDLEY, C. A. AND LA FORGE, M.: The protective action of various agents against chloroform-epinephrine ventricular fibrillation. *J. Pharmacol. & Exper. Therap.*, **95**: 312-317, 1949.
90. HULPIEU, H. R., COLE, V. V. AND VIEIRA, Z.: Blood levels of procaine and p-aminobenzoic acid following the use of intravenous procaine hydrochloride in general anesthesia. *Anesthesiology*, **11**: 333-341, 1950.
91. IGLAUER, A., DAVIS, D. AND ABTSCHULE, M. D.: Auricular fibrillation in normal, intact animals after the intravenous injection of mecholyl (acetyl- β -methylcholine). *Am. Heart J.*, **22**: 47-55, 1941.

92. ING, H. R., DAWES, G. S. AND WAJDA, L.: Synthetic substitutes for atropine. *J. Pharmacol. & Exper. Therap.*, **85**: 85-102, 1945.
93. JACOB, J., MONTÉZIN, G. AND BOYET, D.: Propriétés cardiotoxiques du 2480F (4-amino-4'-diéthylamino-propylamino-diphénylsulfone) Action antagoniste du 1262F (dacrène). *Arch. internat. de pharmacodyn. et de therap.*, **80**: 420-443, 1949.
94. JOHNSON, S. R.: The mechanism of hyperglycemia during anesthesia: an experimental study. *Anesthesiology*, **10**: 379-386, 1949.
95. KALMANSON, R. W. AND SAMPSON, J. J.: Studies of plasma quinidine content. *Circulation*, **1**: 564-575, 1950.
96. KEOGH, P. AND SHAW, F. H.: The pharmacology and toxicity of alstonia alkaloids. *Australian J. Exper. Biol. & M. Sc.*, **21**: 183-6, 1943.
97. KRAYER, O.: Studies on veratrum alkaloids. VIII. Veratramine, an antagonist to the cardioaccelerator action of epinephrine. *J. Pharmacol. & Exper. Therap.*, **96**: 422-437, 1949.
98. KRAYER, O.: Studies on veratrum alkaloids. IX. The inhibition by veratrosine of the cardioaccelerator action of epinephrine and of norepinephrine. *J. Pharmacol. & Exper. Therap.*, **97**: 256-265, 1949.
99. KRAYER, O.: Studies on veratrum alkaloids. XII. A quantitative comparison of the antiaccelerator cardiac action of veratramine, veratrosine, jervine and pseudojervine. *J. Pharmacol. & Exper. Therap.*, **98**: 427-436, 1950.
100. KRAYER, O.: The antiaccelerator cardiac action of quinine and quinidine. *J. Pharmacol. & Exper. Therap.*, **100**: 146-150, 1950.
101. KRAYER, O. AND ACHESON, G. H.: The pharmacology of the veratrum alkaloids. *Physiol. Rev.*, **26**: 383-446, 1946.
102. KRAYER, O. AND BRIGGS, L. H.: Studies on solanum alkaloids. *Brit. J. Pharmacol.*, **5**: 118-124, 517-525, 1950.
103. KRAYER, O. AND VAN MAANEN, E. F.: Studies on veratrum alkaloids X. The inhibition by veratramine of the positive chronotropic effect of accelerans stimulation and of norepinephrine. *J. Pharmacol. & Exper. Therap.*, **97**: 301-307, 1949.
104. KRAYER, O., UHLE, F. C. AND OUBRISSON, P.: Studies on veratrum alkaloids XIV. The antiaccelerator cardiac action of derivatives of veratramine and jervine and of synthetic steroid secondary alkalamines obtained from pregnenolone and from sapogenins. *J. Pharmacol. & Exper. Therap.*, **102**: 261-268, 1951.
105. KRUTA, V. L.: Sur l'activité automatique de l'oreillette gauche isolée du cœur de mammifère. *Arch. internat. de Physiol.*, **40**: 140-157, 1934.
106. LANGENDORFF, O.: Untersuchungen am überlebenden Säugethierherzen. *Pflüg. Arch. f. d. ges. Physiol.*, **61**: 291-332, 1895.
107. LEE, W. V., ORTH, O. S., WANGEMAN, C. P. AND MEEK, W. J.: The mechanism of production of spontaneous cardiac irregularities with high concentrations of cyclopropane. *Anesthesiology*, **4**: 487-496, 1943.
108. LENEL, R., VANLOO, A., ROBBARD, S. AND KATZ, L. N.: Factors involved in the production of paroxysmal ventricular tachycardia induced by epinephrine. *Am. J. Physiol.*, **153**: 553-557, 1948.
109. LEVY, A. G.: Sudden death under light chloroform anaesthesia. *J. Physiol.*, **42**: iii-vii, 1911.
110. LEVY, A. G.: The exciting causes of ventricular fibrillation in animals under chloroform anaesthesia. *Heart*, **4**: 319-378, 1913.
111. LEVY, A. G.: The genesis of ventricular extrasystoles under chloroform; with special reference to consecutive ventricular fibrillation. *Heart*, **5**: 299-334, 1914.
112. LEWIS, T.: The mechanism and graphic registration of the heart beat. Shaw & Sons Ltd., London, 1925.
113. LEWIS, T., DRURY, A. N. AND BULGER, H. A.: Observations upon flutter and fibrillation. VII. The effects of vagal stimulation. *Heart*, **8**: 141-191, 1921.
114. LINENTHAL, A. J., ULICK, S. AND PATTERSON, L. A.: Fluorometric measurement of plasma quinidine and its correlation with cardiac effects in man. *J. Clin. Investigation*, **26**: 1188, 1947.
115. LONG, J. H., OPPENHEIMER, M. J., WESTER, M. R. AND DURANT, T. M.: The effect of intravenous procaine on the heart. *Anesthesiology*, **10**: 406-415, 1949.
116. LORENTE DE NÓ, R. AND FENG, T. P.: Analysis of the effect of barium upon nerve with particular reference to rhythmic activity. *J. Cell. & Comp. Physiol.*, **28**: 397-464, 1946.
117. MACGREGOR, D. F.: The relation of cocaine and of procaine to the sympathetic system. *J. Pharmacol. & Exper. Therap.*, **66**: 393-409, 1939.
118. MARK, L. C., LOTT, W. A., COOPER, J. R. AND BRODIE, B. B.: Studies on diethylaminoethanol. II. Antiarrhythmic activity in two homologous alcohol series. *J. Pharmacol. & Exper. Therap.*, **98**: 405-408, 1950.
119. MARK, L. C., KAYDEN, H. J., MURRAY STREEKE, J., COOPER, J. R., ROVENSTINE, E. A. AND BRODIE, B. B.: The physiological disposition and cardiac effects of procaine amide. *J. Pharmacol. & Exper. Therap.*, **102**: 5-15, 1951.
120. MATTHEWS, B. H. C.: Nerve endings in mammalian muscle. *J. Physiol.*, **78**: 1-53, 1933.
121. MAUTE, F. R.: Reduction of cardiac irritability by the epicardial and systemic administration of drugs as a protection in cardiac surgery. *J. Thoracic Surg.*, **5**: 612-628, 1936.
122. McCRAWLEY, E. L., WESTON, G. A. AND DAVID, N. A.: Evaluation of certain antihistaminics for use in auricular fibrillation. *J. Pharmacol. & Exper. Therap.*, **102**: 250-257, 1951.
123. McMILLAN, T. M. AND WOLFERTH, C. C.: An untoward effect of barium chloride in producing short runs of aberrant ventricular beats. *J. Lab. & Clin. Med.*, **14**: 839-845, 1928-9.
124. MEEK, W. J.: Some cardiac effects of the inhalant anesthetics and the sympathomimetic amines. *Harvey Lect.*, **36**: 188-227, 1941.

125. MEEK, W. J.: Cardiac automaticity and response to blood pressure raising agents during inhalation anesthesia. *Physiol. Rev.*, **21**: 324-336, 1941.
126. MEEK, W. J., HATHAWAY, H. R. AND ORTH, O. S.: The effects of ether, chloroform and cyclopropane on cardiac automaticity. *J. Pharmacol. & Exper. Therap.*, **61**: 245-262, 1937.
127. MILLER, G. H.: The effects of general anesthesia on the vascular activity of the gastro-intestinal tract. A study of ether, chloroform, ethylene and nitrous oxide. *J. Pharmacol. & Exper. Therap.*, **27**: 41-56, 1926.
128. MINER, G. H.: On dynamic equilibrium in the heart. *J. Physiol.*, **46**: 349-383, 1913.
129. MOE, G. K., HARRIS, A. S. AND WIGGERS, C. J.: Analysis of the initiation of fibrillation by electrographic studies. *Am. J. Physiol.*, **140**: 473-492, 1941.
130. MOE, G. K., MALTON, S. D., RENNICK, B. R. AND FREYBURGER, W. A.: The role of arterial pressure in the induction of idioventricular rhythms under cyclopropane anesthesia. *J. Pharmacol. & Exper. Therap.*, **94**: 319-327, 1948.
131. MOE, G. K., HENNEY, E. E., FREYBURGER, W. A. AND MALTON, S. D.: The effect of cyclopropane on cardiac work capacity. *Anesthesiology*, **10**: 706-713, 1949.
132. MOISSET DE ESPANÉS E.: Acción de la fagarina I. Merck y de la quinidina sobre la excitabilidad y capacidad de fibrilación del miocardio. *Rev. Soc. argent. de biol.*, **13**: 112-115, 1937.
133. MOISSET DE ESPANÉS E.: Acción de la fagarina I. Merck sobre la fibrilación ventricular primaria producida por la obstrucción coronaria experimental. *Rev. Soc. argent. de biol.*, **13**: 116-120, 1937.
134. MOISSET DE ESPANÉS E., AND WERSLER, B.: Antifibrillating action of N-methyl-dibenzylamine and some of its derivatives. *Proc. Soc. Exper. Biol. & Med.*, **63**: 195-198, 1946.
135. MORRIS, L. E. AND HAID, B.: Effects of procaine amide on cardiac irregularities during cyclopropane anesthesia. *Anesthesiology*, **12**: 328-339, 1951.
136. MURPHY, Q., CRUMPTON, C. W. AND MEEK, W. J.: The effect of blood pressure rise on the production of cyclopropane-epinephrine induced cardiac irregularities. *Anesthesiology*, **10**: 416-420, 1949.
137. MURPHY, Q., O'BRIEN, G. S. AND MEEK, W. J.: The effects of aliphatic sympathomimetic amines on cardiac automatic tissue in dogs under cyclopropane. *Anesthesiology*, **11**: 437-442, 1950.
138. NAHUM, L. H. AND HOFF, H. E.: The experimental production of ventricular fibrillation, and its prevention by acetyl- β -methylcholine chloride. *Am. J. Physiol.*, **109**: 78-79, 1934.
139. NAHUM, L. H. AND HOFF, H. E.: The mechanism of sudden death in experimental acute benzol poisoning. *J. Pharmacol. & Exper. Therap.*, **56**: 336-345, 1934.
140. NAHUM, L. H. AND HOFF, H. E.: The rôle of adrenaline in the production of ventricular rhythms and their suppression by acetyl- β -methylcholine chloride. *J. Pharmacol. & Exper. Therap.*, **52**: 235-245, 1934.
141. NAHUM, L. H. AND HOFF, H. E.: Auricular fibrillation in hyperthyroid patients. *J. Am. M. A.*, **105**: 254-257, 1935.
142. NICKERSON, M. AND NOMAGUCHI, G.: Mechanism of dibenamine protection against cyclopropane-epinephrine cardiac arrhythmias. *J. Pharmacol. & Exper. Therap.*, **95**: 1-11, 1949.
143. NICKERSON, M. AND SMITH, S. M.: Protection against cyclopropane-epinephrine arrhythmias by dibenamine and other agents. *Anesthesiology*, **10**: 562-576, 1949.
144. NOTH, P. H., ESSEX, H. E. AND BARNES, A. R.: The effect of the intravenous injection of acetylcholine on the electrocardiogram of the dog. *Proc. Staff Meet. Mayo Clin.*, **14**: 348-357, 1939.
145. ORTH, O. S. AND RITCHIE, G.: A pharmacological evaluation of dihydroergotamine methanesulfonate (D. H. E. 45). *J. Pharmacol. & Exper. Therap.*, **90**: 166-173, 1947.
146. PAPILIAN, V., RUSCU, I. G. AND ANTONESCU, C.: Le sympathique et la syncope adrénalino-chloroformique. *Compt. rend. Soc. biol.*, **118**: 471-472, 1935.
147. PRINZMETAL, M., CORDAY, E., BRILL, I. C., SELLERS, A. L., OBLATH, R. W., FLIEG, W. A. AND KRUGER, H. E.: Mechanism of the auricular arrhythmias. *Circulation*, **1**: 241-245, 1950.
148. PRINZMETAL, M., CORDAY, E., OBLATH, R. W., KRUGER, H. E., BRILL, I. C., FIELDS, J., KENNAMER, S. R., OSBORNE, J. A., SMITH, L. A., SELLERS, A. L., FLIEG, W. AND FINSTON, E.: Auricular flutter. *Am. J. Med.*, **11**: 410-430, 1951.
149. RENNICK, B. R., PARDO, E. G., GRUNZIT, C. C. AND MOE, G. K.: The role of thoracic sympathetic pathways in the induction of ventricular ectopic rhythms by epinephrine and cyclopropane. *J. Pharmacol. & Exper. Therap.*, **101**: 176-180, 1951.
150. RESNIK, W. H.: Observations on the effect of anoxemia on the heart. *J. Clin. Investigation*, **2**: 125-141, 1925.
151. ROBBINS, B. H. AND BAXTER, J. H.: Studies of cyclopropane III. The relation of electrocardiographic changes to the arterial concentrations of oxygen, carbon dioxide and cyclopropane in dogs anesthetized with cyclopropane. *J. Pharmacol. & Exper. Therap.*, **61**: 162-174, 1937.
152. ROBBINS, B. H., FITZHUUGH, O. G. AND BAXTER, J. H.: Studies of cyclopropane VII. An analysis of the factors controlling the heart rate in dogs anesthetized with cyclopropane or ether after premedication with morphine. *J. Pharmacol. & Exper. Therap.*, **66**: 206-215, 1939.
153. ROBINSON, G. C.: The influence of the vagus nerves on the faradized auricles in the dog's heart. *J. Exper. Med.*, **17**: 429-443, 1913.
154. ROSENBERG, B., KAYDEN, H. J., LIEF, P. A., MARK, L. C., MURRAY STEELE, J. AND BRODIE, B. B.: Studies on diethylaminoethanol. I. Physiological disposition and action on cardiac arrhythmias. *J. Pharmacol. & Exper. Therap.*, **95**: 18-27, 1949.
155. ROSENBLUTH, A. AND GARCÍA RAMOS, J.: Estudios sobre el flutter y la fibrilación. IV. La naturaleza del flutter auricular y de la actividad lenta autosostenida del músculo auricular aislado. *Arch. Inst. Cardiol. Mexico*, **17**: 441-457, 1947.

136. ROSENBLUTH, A. AND WIENER, N.: The mathematical formulation of the problem of conduction of impulses in a network of connected excitable elements, specifically in cardiac muscle. *Arch. Inst. Cardiol. Mexico*, **16**: 205-265, 1946.
137. ROTHBERGER, C. J. AND SACIS, A.: Rhythmicity and automatism in the mammalian left auricle. *Quart. J. Exper. Physiol.*, **29**: 69-81, 1939.
138. ROTHBERGER, C. J. AND WINTERBERG, M.: Über scheinbare Vaguslähmung (bei Muskarin, Physostigmin und anderen Giften sowie bei intrakardialer Drucksteigerung). *Pflüg. Arch. ges. Physiol.*, **132**: 233-254, 1919.
139. ROTHBERGER, C. J. AND WINTERBERG, H.: Über die experimentelle Erzeugung extrasystolischer ventrikulärer Tachykardie durch Acceleransreizung. *Pflüg. Arch. ges. Physiol.*, **142**: 461-530, 1911.
140. ROY S. DE: L'activité automatique rythmique de l'oreillette gauche est due à l'intervention des "substances actives" d'origine nodale. *Arch. internat. de Physiol.*, **43**: 299-315, 1936.
141. SAMAN, A.: The effect of adrenaline, atropine and ether on the heart rate of normal dogs and of animals deprived of different parts of the autonomic nervous system. *Arch. internat. de pharmacodyn. et de thérap.*, **50**: 101-127, 1935.
142. SCHILLONG, F.: Untersuchungen über die Grundeigenschaften des Herzmuskels und ihre Beziehungen zueinander. *Ztschr. f. Biol.*, **82**: 451-458, 1925.
143. SCHERF, D.: Untersuchungen über die Entstehungsweise der extrasystolen und der extrasystolischen allorhythmien. *Ztschr. f. d. ges. exper. Med.*, **65**: 198-275, 1929.
144. SCHERF, D.: Studies on auricular tachycardia caused by aconitine administration. *Proc. Soc. Exper. Biol. & Med.*, **64**: 233-239, 1947.
145. SCHERF, D.: Effect of fagarine on auricular fibrillation. *Proc. Soc. Exper. Biol. & Med.*, **67**: 59-60, 1948.
146. SCHERF, D. AND CHICK, F. B.: Abnormal cardiac rhythms caused by acetylcholine. *Circulation*, **3**: 764-769, 1951.
147. SCHERF, D. AND CHICK, F. B.: Experimental parasymplole. *Am. Heart J.*, **42**: 212-225, 1951.
148. SCHERF, D. AND ROMANO, F. J.: Extrasystoles in groups. *Am. Heart J.*, **35**: 81-93, 1948.
149. SCHERF, D., ROMANO, F. J. AND TERRANOVA, R.: Experimental studies on auricular flutter and auricular fibrillation. *Am. Heart J.*, **36**: 241-251, 1948.
150. SCHERF, D., SCHARF, M. M. AND GÖKLEN, M. F.: Effect of stretch and pressure on stimulus formation in the dog's auricle. *Proc. Soc. Exper. Biol. & Med.*, **70**: 706-711, 1949.
151. SCHERF, D., SILVER, A. M. AND WEINBERG, L. D.: Clinical observations with fagarine. *Ann. Int. Med.*, **30**: 100-120, 1949.
152. SCHERF, D. AND TERRANOVA, R.: Mechanism of auricular flutter and fibrillation. *Am. J. Physiol.*, **139**: 137-142, 1949.
153. SCOTT, C. C., ANDERSON, R. C. AND CHEN, K. K.: Comparison of the pharmacologic action of quinidine and dihydroquinidine. *J. Pharmacol. & Exper. Therap.*, **84**: 184-188, 1945.
154. SEGERS, M.: Mémoires Acad. Méd. Belg., **1**: 1-30, 1941.
155. SEGERS, M.: Les mécanismes des réglages de l'amplitude des contractions cardiaques. *Arch. internat. de physiol.*, **52**: 291-348, 1942.
156. SEGERS, M.: Le battement auto-entretenu du cœur. *Arch. internat. de pharmacodyn. et de thérap.*, **75**: 144-156, 1947.
157. SHEN, T. C. R.: The protective action of piperido-methyl-3-benzodioxane (F.933), diethyl-amino-methyl-3-benzodioxane (F.883) and yohimbine upon the chloroform-adrenaline ventricular fibrillation. *Arch. internat. de pharmacodyn. et de thérap.*, **59**: 243-251, 1938.
158. SHEN, T. C. R.: Benzol-adrenaline cardio-ventricular fibrillation and methods of prevention. *Arch. internat. de pharmacodyn. et de thérap.*, **61**: 43-59, 1939.
159. SHEN, T. C. R. AND MARRE, R.: Further studies on cardioventricular fibrillation. *Arch. internat. de pharmacodyn. et de thérap.*, **64**: 55-78, 1940.
160. SHEN, T. C. R. AND SIMON, M. A.: The protecting action of novocaine upon chloroform-adrenalin ventricular fibrillation. *Arch. int. Pharmacodyn.*, **59**: 68-74, 1938.
161. SHIDEMAN, F. E. AND PROCTA, L.: The pharmacology of the monomethyl ethers of mono-, di- and tripropylene glycol in the dog with observations on the auricular fibrillation produced by these compounds. *J. Pharmacol. & Exper. Therap.*, **102**: 79-87, 1951.
162. SHOOKHOFF, CH.: Zur Kenntnis der Wirkung von Novocain, bez. Cocain auf des Säugetierherz. *Ztschr. f. d. ges. Med.*, **49**: 110-123, 1926.
163. SMITH, J. R. AND WILSON, K. S.: Studies on the production and maintenance of experimental auricular fibrillation. *Am. Heart J.*, **27**: 176-185, 1944.
164. SMITH, P. K., WINKLER, A. W. AND HOFF, H. E.: Cardiovascular changes following the intravenous administration of barium chloride. *J. Pharmacol. & Exper. Therap.*, **68**: 113-122, 1940.
165. SOKOLOV, M. AND EDGAR, A. L.: Blood quinidine concentrations as a guide in the treatment of cardiac arrhythmias. *Circulation*, **1**: 576-592, 1950.
166. STUTZMAN, J. W. AND ALLEN, C. R.: Adrenolytic action of cyclopropane. *Proc. Soc. Exper. Biol. & Med.*, **47**: 218-222, 1941.
167. STUTZMAN, J. W., MURPHY, Q., ALLEN, C. R. AND MEER, W. J.: Further studies on the production of cyclopropane-epinephrine tachycardia. *Anesthesiology*, **8**: 579-583, 1947.
168. STUTZMAN, J. W. AND PETTINGER, F. L.: Mechanism of cardiac arrhythmias during cyclopropane anesthesia. *Anesthesiology*, **10**: 374-378, 1949.
169. TAQUINI, A. C.: Fagarine—a new drug for the treatment of fibrillation and flutter. *Am. Heart J.*, **33**: 719, 1947.

190. TAYLOR, I. B., STEARNS, A. B., KURTZ, H. C., HENDERSON, J. C., SIGLER, L. E. AND NOLTE, E. C.: Intravenous procaine—an adjuvant to general anesthesia: a preliminary report. *Anesthesiology*, **11**: 185-198, 1950.
191. THIENES, C. H., GREELEY, P. O. AND GUEDEL, A. E.: Cardiac arrhythmias under cyclopropane anesthesia. *Anesthesiology*, **2**: 611-620, 1941.
192. TOURNADE, A., MALMÉJAC, J. AND DJOURNO, A.: Par quel mécanisme la syncope chloroformique protège-t-elle d'ordinaire contre la syncope adrénalino-chloroformique? *Compt. rend. Soc. biol.*, **110**: 540-542, 1932.
193. TOURNADE, A. AND RAYMOND-HAMET.: Syncope nor adrénalino-chloroformique. *Compt. rend. Soc. biol.*, **111**: 897-900, 1932.
194. TREVAN, J. W. AND BOOCK, E.: The relation of hydrogen ion concentration to the action of the local anaesthetics. *Brit. J. Exper. Path.*, **8**: 307-315, 1927.
195. TRIFOD, J.: Fibrillation cardiaque et activité antifibrillante sur le cœur isolé de mammifère. *Arch. internat. de pharmacodyn. et de thérap.*, **85**: 121-128, 1951.
196. VAN DONGEN, K.: The action of some drugs on fibrillation of the heart. *Arch. internat. de pharmacodyn. et de thérap.*, **83**: 80-104, 1936.
197. VAN DONGEN, K.: The action of novocaine on fibrillation of the heart. *Arch. internat. de pharmacodyn. et de thérap.*, **60**: 206-208, 1938.
198. VAN DONGEN, K.: The action of F933 (piperidomethyl-3 benzodioxane) on fibrillation of the heart. *Arch. internat. de pharmacodyn. et de thérap.*, **63**: 88-89, 1939.
199. WASTL, H.: Die übernormale Phase der Erhöhung des Herzmuskels nach einer Systole. *Ztschr. f. Biol.*, **75**: 289-292, 1922.
200. WEDD, A. M.: The influence of rate and temperature change and of various cardiac drugs on rhythmicity, contractility, and the refractory period of the turtle heart. *Am. J. Physiol.*, **108**: 265-269, 1934.
201. WÉGRIA, R. AND BOYLE, M. N.: Correlation between the effect of quinidine sulfate on the heart and its concentration in the blood plasma. *Am. J. Med.*, **4**: 373-382, 1948.
202. WÉGRIA, R. AND NICKERSON, N. D.: The effect of papaverine, epinephrine and quinidine on the fibrillation threshold of the mammalian ventricles. *J. Pharmacol. & Exper. Therap.*, **75**: 50-57, 1942.
203. WÉGRIA, R. AND NICKERSON, N. D.: The benzol-adrenalin test as a reliable method of estimating changes in the sensitivity of the dog's ventricles to fibrillation. Application of the method to the study of quinidine sulfate. *Am. Heart J.*, **25**: 58-63, 1943.
204. WÉGRIA, R. AND WIGGERS, C. J.: Factors determining the production of ventricular fibrillation by direct currents (with a note on chronaxie). *Am. J. Physiol.*, **131**: 104-118, 1940.
205. WÉGRIA, R. AND WIGGERS, C. J.: Production of ventricular fibrillation by alternating currents. *Am. J. Physiol.*, **131**: 119-128, 1940.
206. WEIDMANN, S.: Effect of current flow on the membrane potential of cardiac muscle. *J. Physiol.*, **115**: 227-236, 1951.
207. WHITTERIDGE, D. AND BULBRING, E.: Changes in activity of pulmonary receptors in anaesthesia and their influence on respiratory behaviour. *J. Pharmacol. & Exper. Therap.*, **81**: 340-359, 1944.
208. WIGGERS, C. J.: The mechanism and nature of ventricular fibrillation. *Am. Heart J.*, **20**: 399-412, 1940.
209. WIGGERS, C. J. AND WÉGRIA, R.: Ventricular fibrillation due to single, localized induction and condenser shocks applied during the vulnerable phase of ventricular systole. *Am. J. Physiol.*, **128**: 500-505, 1939.
210. WIGGERS, C. J. AND WÉGRIA, R.: Quantitative measurement of the fibrillation thresholds of the mammalian ventricles with observations on the effect of procaine. *Am. J. Physiol.*, **131**: 296-308, 1940.
211. WIGGERS, C. J., WÉGRIA, R. AND PISERA, B.: The effects of myocardial ischemia on the fibrillation threshold—the mechanism of spontaneous ventricular fibrillation following coronary occlusion. *Am. J. Physiol.*, **131**: 309-316, 1940.
212. WOODBURY, L. A., HECHT, H. H. AND CHRISTOPHERSON, A. R.: Membrane resting and action potentials of single cardiac muscle fibers of the frog ventricle. *Am. J. Physiol.*, **164**: 307-318, 1951.
213. WOODBURY, L. A., WOODBURY, J. W. AND HECHT, H. H.: Membrane resting and action potentials of single cardiac muscle fibers. *Circulation*, **1**: 264-266, 1950.
214. YOUNG, W. B., WANGEMAN, C. P., GRISWOLD, H. E. AND KARSTENS, A. I.: Effect of cyclopropane anesthesia on the glucose and epinephrine levels of the blood. *Anesthesiology*, **4**: 31-35, 1943.

THE DOSAGE SCHEDULE OF CHEMOTHERAPEUTIC AGENTS

E. K. MARSHALL, JR.

Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University.

We shall define a chemotherapeutic agent as a drug used in treatment of a systemic infection. Although Ehrlich, in addition, limited the term to drugs of known chemical constitution in order to exclude antitoxins, serums and vaccines, we shall not adopt this restriction since antiparasitic agents of microbial origin which may be of unknown constitution at present play an important role in therapy.

In the investigation and use of any chemotherapeutic agent, there is a triad of three factors which must be taken into account. These are the parasite, the host, and the drug. It is the interplay of these three factors which accounts for the cure of the infection. An understanding of these factors demands not only a study of the effect of the drug on the parasite *in vitro* and *in vivo*, but also an investigation of the absorption, excretion, degradation and distribution of the drug in the host organism.

In designing the most satisfactory dosage schedule for any chemotherapeutic agent at least three factors must be considered in connection with the effect of the drug on the parasite *in vivo*. These are the total dosage, the duration of treatment, and the interval between administration of individual doses. In any type of treatment an unlimited number of variations with respect to total dosage, duration of treatment, and interval between doses are possible. Unless experiments are designed properly, one cannot be certain whether total dosage, duration of treatment, or spacing of the individual doses is responsible for the better therapeutic effect.

The interval between doses should be determined by knowledge of whether or not constant contact between chemotherapeutic agent and parasite is necessary for most effective therapy and by a knowledge of the absorption, distribution, excretion and degradation in the host of the particular drug in question. It would appear that each individual drug, and possibly each infection for which it is used, must be studied in order to determine whether or not a dosage schedule which attempts to maintain a constant concentration of drug in contact with the parasite is necessary for the most effective therapy. Spacing the total dosage may involve a large initial loading dose followed by smaller maintenance doses. This may be necessary for one of two different reasons; namely, to establish rapidly equilibrium in the body with the drug or to protect from the infection until subsequent doses can be given. The large initial doses recommended with the sulfonamides and quinacrine are examples of the former, while a large initial dose of penicillin in a mouse infection (109) serves as an example of the latter.

The evidence appears very strong that in certain instances most effective therapy is obtained by rapidly attaining and then maintaining an effective concentration of drug in blood for a certain time, while with other drugs just as efficient or even more efficient therapy may be obtained when the concentration of drug

in the blood is not maintained constant but is allowed to rise to a peak and then fall to a negligible level before the next dose of drug is given. It will be shown that sulfonamides are most effective when given by continuous dosage while penicillin is just as effective in the same infections when given intermittently. One might explain this difference by assuming the sulfonamides to be more loosely bound to receptors in bacteria than penicillin (for discussion, see Marshall, 57). It should be pointed out that *in vitro* studies alone can never serve as a basis for devising the most efficient dosage schedule, but in addition it is necessary to have knowledge of the behavior of the drug in the host, and direct studies of dosage schedules in infected animals. Then, trial in diseased human beings can be undertaken to see if a dosage schedule, based on the knowledge obtained, is satisfactory. In patients, investigation of dosage schedules is much more difficult and less accurate than in rigidly controlled experiments on infected animals. There are added difficulties in patients with a disease having a high mortality. If some dosage schedule has been tried on patients and found more or less satisfactory, it is very difficult to attempt to modify it.

In 1917, Moore and Chesney (69) in a study of the value of ethylhydrocuprein in pneumonia in man utilized the acquired bactericidal power of serum to determine the best dosage schedule. Two quotations will be given from their paper as their dosage schedule of this drug resembles very closely the schedule adopted for the sulfonamides but antedates that schedule by more than two decades. They state: "... what is the optimum method of administration of the drug, both as regards the route, and the regulation of the size and spacing of the individual doses, as determined by a study of this acquired bactericidal property of the serum?" "... in order to destroy pneumococci within the living animal, the drug should be repeatedly administered in suitable doses, at comparatively short intervals of time, in order that the pneumococidal action in the circulating fluids may be continuously preserved for the necessary length of time." It may be noted that in the dosage schedule used by Moore and Chesney a large dose was first given to attain quickly a bactericidal action of serum and then smaller doses were given at frequent intervals in order to maintain this bactericidal action. The use of both priming and maintenance doses has been later found necessary for many chemotherapeutic agents. It is interesting that these authors did not write of the ethylhydrocuprein concentration in blood but used the term "acquired bactericidal property," which described exactly what they were measuring. This was in contrast to what happened with penicillin, where results of drug concentration in blood obtained by a non-specific, easily influenced biological method were used as if they were obtained by a highly specific and accurate chemical method. In this study of ethylhydrocuprein, no evidence was available that the maintenance of a bactericidal property of serum was essential for the most effective method for use of the drug. However, even today, unless evidence were available to the contrary, the dosage schedule used by Moore and Chesney would appear to be the one of choice as a first attempt to discover the value of a chemotherapeutic drug. Subsequent investigation would then be made to determine the optimal dosage schedule.

We can now consider the dosage schedule of arsenic and antimony compounds. Ehrlich attempted a *therapia sterilisans magna* where one dose of drug would completely eradicate the infection. When this failed with arsphenamine in human syphilis, the dosage of this arsenic compound was given in a more or less empirical manner and not based on any carefully controlled experimental study. Only twenty to thirty years after their introduction, do we find a change in dosage schedule of the arsenicals. Hyman *et al.* (46) introduced the so-called massive dose chemotherapy for syphilis by the intravenous drip method, where over only a five day period a large dose of neoarsphenamine (and later oxophenarsine) was given by slow intravenous drip. This 5-day treatment of early syphilis was apparently quite effective and nullified the syphilographer's idea of the necessity for treatment for more than a year. However, the short intensive method proved to be disadvantageous from the standpoint of toxicity. The published results did stimulate Eagle and Hogan to undertake an experimental study in syphilitic rabbits of the dosage schedule of oxophenarsine (30). From their studies, it appears that the curative total dose of oxophenarsine in rabbit syphilis does not vary greatly despite wide variations in the duration of treatment and frequency of injections. The data presented in support of this conclusion are not too impressive and one cannot accept the author's statement that the data prove that a high concentration of drug in body fluids for a short period of time was more effective therapeutically than a low concentration maintained for a relatively long period. This work of Eagle and Hogan does not contain a single experiment in which the concentration of drug in blood was maintained for more than a fraction of the twenty-four hours. The final evaluation of "cure" at six months was only made on a proportion of the animals used. The correction used on animals followed for only six weeks is subject to a large error. In addition, there are very large probable errors in data of this kind with the small number of animals used. The authors did not seem to appreciate this and made no attempt to estimate these errors.

It has also been found that the curative dose of reduced trypanamide for a trypanosome infection in mice is the same for intravenous, subcutaneous, and intraperitoneal injection. From these data, it is concluded that a relatively high concentration of drug for a short period is equally as effective as a relatively low concentration for a longer period (42). It is probable that with the organic arsenicals in the therapy of syphilis and of trypanosomiasis, the total dosage is more important than the manner in which the dosage is divided. This means that a constantly maintained concentration of drug in the blood is no more effective than allowing intervals during treatment where the drug has disappeared from the blood.

Vianna (103) in 1912 discovered the curative effect of antimony in mucocutaneous leishmaniasis, and Di Cristina and Caronia (24) three years later found a marked reduction of mortality in kala-azar by administering tartar emetic by intravenous injection on alternate days. The practical consequences of this discovery on a disease which hitherto had a mortality of 95 per cent were extraordinary. Although a cure rate of over 80 per cent was attained, the imperfections of

the lengthy tartar emetic treatment led to the search for a more intensive and less unpleasant form of treatment. The pentavalent antimonials made it possible to reduce the number of injections from 30 to 10, but treatment was still given on alternate days. The toxicity of the preparations used apparently conditioned the dosage schedule, as daily injections were tried when less toxic preparations became available (37). In the experimental disease in the hamster, it has been found that injections of pentavalent antimonials given daily were less effective than an equal number given bi-weekly, the total dosage remaining constant (104). However, in both the human and the experimental disease, variation in the number of injections has been accompanied by variation in the duration of treatment. Stilbamidine, which has proved effective in the case of antimony-resistant kala-azar, has been given intravenously daily or even at less frequent intervals. However, the total dosage, the number of injections and the duration of treatment have all been varied (86).

The non-metallic and extremely interesting compound suramin, which has been shown to be highly effective in the early stages of trypanosomiasis is retained in the body for such a long time that dosage can be given at very long intervals. In fact, a single intravenous dose is claimed to protect for several months. There is no mystery about this prolonged action, as the drug remains in the blood for many months (38).

There are three features in connection with the sulfonamides which were responsible for the development of rapid, accurate, and fairly specific methods for their chemical determination in body fluids and tissues (60, 41, 55, 9, 106). These are the use of relatively large dosage, the nature of the distribution of these drugs in the organism, and the fact that all active drugs of this group possess an aryl-amino-group or are converted in the body to a compound containing this group. Such compounds containing an aryl-amino-group can easily be diazotized and coupled to form a highly colored azo-dye. The use of these simple methods for the determination of sulfonamides led to the development of the dosage schedule necessary to obtain the optimal therapeutic response. The concepts which have guided therapy with this class of drugs are a) the use of the concentration of drug in blood or plasma rather than dosage by mouth to control therapeutic response, and b) the demonstration that the best therapeutic effect is obtained by raising the drug concentration in blood to the desired level rapidly by giving a large loading dose and then giving the drug at frequent intervals, day and night, so as to maintain this concentration in the blood for several days. Let us now inquire into the evidence for the validity of these concepts.

In regard to the first, it may be said that the sulfonamides which have had clinical use have an apparent distribution of from 45 to 80 per cent of the body weight. This means that within a factor of two, at most, the drug concentration in plasma is proportional to the total amount of drug in the body with the various sulfonamides. Since some of these drugs are incompletely and erratically absorbed from the gastrointestinal tract and different ones are excreted at different rates by the kidney, the amount of drug in the body is represented better by its concentration in plasma or blood than by dosage by mouth. Granting that therapeutic

tic activity is related to the amount of drug in the body (exclusive of that in the gastrointestinal tract), it follows that for these drugs the concentration in blood should correlate with chemotherapeutic activity. In fact, soon after their introduction, sulfonamides were used in therapy on the basis of concentration in blood rather than on dosage administered.

In regard to the second concept of the necessity for maintaining a more or less constant concentration of drug in the blood in order to obtain the optimal therapeutic response, it must be admitted that published data are rather scanty in support of this thesis. However, there is little doubt that it is, on the whole, correct. In pneumococcal infections of mice, sulfanilamide was stated by the earlier workers (12, 50, 34) to have no beneficial effect. However, in all the above experiments doses of 5 to 25 mgm. per mouse were given once a day. Schmidt (81, 82) found that if 5 or 10 mgm. of sulfanilamide were given every 4 to 6 hours, a marked curative effect was observed on many types of pneumococcal infections of mice. Litchfield, White and Marshall (48) also found sulfanilamide very effective in a pneumococcal infection of mice when the drug was administered by the drug-diet method. Also it was found that 4,4'-diaminodiphenylsulfone was twenty to one hundred times as active as sulfanilamide in a streptococcal infection of mice when the drugs were given once a day, but it was only about three times as effective when the drugs were administered by the drug-diet method. The difference appears to be due to the slower absorption and excretion of the sulfone as compared to sulfanilamide (61). It has also been found that in an infection of mice with *E. coli*, sulfathiazole administered in 5 doses (at 2-hour intervals) was more effective than a single dose, or, in other words, the long duration of a low concentration of drug in blood is more effective than a short duration of a high concentration (108). In addition, in lophurae malaria in the duck, the sulfonamides are much more effective therapeutically when the concentration in blood is maintained more or less constant than when intermittent doses are given.

Thus, after the sulfonamides had been established as effective agents in bacterial infections, a number of clinical and experimental studies were undertaken to test their efficacy in malaria. These were contradictory partly on account of a species difference in the susceptibility of parasites and partly due to the different dosage schedules which were used. It was found that the maintenance of a constant concentration of a sulfonamide in the blood for a sufficient length of time is just as important for effective therapy of lophurae malaria in the duck as it is in bacterial chemotherapy. When these drugs were administered to ducks with lophurae malaria by the drug-diet method so as to maintain a more or less constant concentration of drug in the blood, it was found in every case that more drug was required to produce the same therapeutic response when given as a single daily dose than when administered in the diet. Thus 2.6 times as much sulfanilamide, 10.5 times as much sulfaguandine, and more than 40 times as much sulfathiazole were required with single doses per day as were needed with continuous administration (62).

For a long time the only effective chemotherapeutic agent for use in malaria was quinine. Usually, it was given at first 1 or 3 times a day, but occasionally was

administered so as to try and maintain the amount of alkaloid in the body as constant as possible. Thus, MacGilchrist (51) in comparing the relative therapeutic value of several cinchona alkaloids in malaria gave a dose of medicine every eight hours throughout the twenty-four hours of the day so as to keep the amount of alkaloid in the body as nearly constant as possible. Others (26) have given doses of quinine every three hours from 6:00 A.M. to 9:00 P.M. with apparently the same object in view. The reason for attempting to maintain a more or less constant concentration of drug in the blood in treating malaria may have been the desire to have an adequate concentration present at sporulation if this was irregular as a result of several superimposed infections. The belief that quinine is effective only during sporulation was widely held by many, but others held the dogma that quinine is only effective on young schizonts. There was little or no scientific evidence available on the question.

Experimental malarial infections with avian parasites were used for testing the efficacy of new drugs in 1926. The dosage schedule in common usage for many years was that of Roehl (80), *i.e.*, the administration *per os* of one dose of drug per day for 6 days. There was no information as to whether this was the optimal method of therapy. It is of interest to recall that in 1914 Marks (53) had found that methylene blue was therapeutically effective when fed in the diet but ineffective when given in single daily dosage in an avian malarial infection. Presumably, the diet method prolonged the persistence of the drug in the blood.

Quinine is more effective when given in a single daily dose than in divided doses or by continuous intravenous injection in lophurae malaria of the duck (58, 22, 8). However, the reverse is true in cynomolgi malaria in the monkey where it has been shown that 4 doses per day for 7 days are three times as effective as a daily dose for the same time (13). It should be noted that in lophurae malaria the effectiveness was based on reduction of parasitemia, while in cynomolgi malaria cure of the disease was the criterion used. This shows clearly that not only the particular drug but the species of parasite and probably the host must be taken into consideration before generalizations as to dosage schedules can be made. Obviously, a more or less constant concentration of drug in the blood is not always the most effective method of therapy in all malarias and with all drugs.

In the elaborate search for new antimalarial drugs carried out in this country during World War II (93), it was assumed for human malarias from analogy to the sulfonamides that 1) the antimalarial action of a drug is related to its concentration in the plasma, and 2) that a dosage schedule giving a more or less constant concentration of drug in plasma furnishes the most effective therapy (89, 2). However, there is no unequivocal evidence that either of these assumptions is true for human malaria for all drugs. Let us consider dosage schedules of the two drugs which have been used and studied most in human malarias, namely, quinine and quinaerine. These two drugs differ markedly in their physiological disposition. Quinine is accumulated in the tissues to a very limited extent and is rapidly excreted and degraded. On the other hand, quinaerine is highly localized in the tissues, many tissues containing several thousand times the concentration present in plasma, and persists in the organism for a long time. It appears that

in the case of quinine, the concentration of drug in the plasma represents fairly accurately the amount of drug present in the organism, while in the case of quinacrine the concentration of drug in plasma can vary widely with a constant total amount of drug in the body. In fact, the total amount of quinacrine present in plasma is extremely minute compared to the total amount in the body.

In the duck, it has been found that with a constant dosage, the concentration of quinacrine in the plasma is highly variable in different animals or in the same animal at different times. These variations in concentrations of drug in plasma are caused mainly by variations in the distribution ratio of quinacrine between plasma and tissues and not by variations in the total amount of drug in the organisms, as is the case with the sulfonamides. Moreover, it has been shown that variations of concentration of drug in plasma are not correlated with the concentration of drug in erythrocytes or in the malarial parasites. In the duck antimalarial activity is well correlated with the dosage of quinacrine and with the total amount of drug in the host, but very poorly or not at all correlated with the drug concentration in plasma (59).

In man there are no data on variations in the distribution ratio of quinacrine between plasma and tissues; it has been assumed in man that the concentration of quinacrine in the plasma is correlated with the concentration of quinacrine in tissues and with therapeutic activity (90, 25, 33, 87). However, in the case of the rabbit and of the dog it has been shown that variations in concentrations of quinacrine in plasma produced by the same dosage are not correlated with concentrations of drug in the tissues; here, as in the duck, the distribution ratio between plasma and tissues is highly variable (23). In addition, when quinacrine is used to treat blood-induced infections of cynomolgus malaria in the monkey, it has been found that the percentage of cures is directly proportional to the dosage given but is not at all correlated with variations in the concentrations of drug in plasma produced by the same dose (83).

In the case of quinine, therapeutic activity in vivax malaria appears to be correlated with variations in concentrations of drug in plasma produced by the same dosage (88). Quinine, although apparently concentrated to some extent in tissues, resembles the sulfonamides more than it does quinacrine in its distribution pattern.

Quinine was usually given before World War II on a dosage schedule of three times a day for therapeutic effect and once a day for suppressive action. The therapeutic dose may have been split in order to avoid toxicity from a large single dose. In studies during the War, it was given so as to achieve a fairly stable concentration in the plasma (94). There is however, no evidence that I know of, to show that in human malarias a fairly constant concentration of quinine in plasma is more or less effective than concentrations which fall below the effective level for a considerable period of time. The dosage schedule recommended today is the administration of a dose three times daily (18).

The dosage schedule proposed and used for quinacrine until World War II was administration of 0.1 gram three times a day. Its action in controlling an attack of malaria was found to be slower than that of quinine. Bryant (10) in 1942

published observations which he had made in East Africa for several years before the War. Noting that the fever decreased rapidly when the urine became bright yellow (third or fourth day of quinacrine administration) and believing this to be due to the exceeding of a renal threshold of the drug in plasma, he administered large doses of quinacrine on the first and second days of treatment. He found that the urine became yellow and the fever receded on the first or second day. Findlay *et al.* (39) also used loading doses of quinacrine in treating malaria in West Africa partly on an empirical basis and partly as a result of Bryant's work. Shannon *et al.* (90) before the observations of Bryant were known made a careful study of the pharmacology of quinacrine. As a result of this study, they proposed an entire revision of the dosage schedule of quinacrine for most efficient therapeutic and suppressive therapy. This is the dosage schedule recommended for quinacrine today, a large loading dose and a maintenance dose thereafter (18).

When penicillin¹ was introduced for the treatment of bacterial infections, it was assumed that most effective therapy required the maintenance of a constant concentration of drug in plasma, day and night, for a sufficient length of time. There was no evidence at all that this idea was correct; it was, indeed, a transference to penicillin of a concept which had been established with the sulfonamides. Due to the extremely rapid excretion of penicillin by the kidneys, it was at first thought necessary to give a continuous intravenous injection of the drug to attain effective therapy. However, soon after its introduction, the continuous intravenous drip was abandoned in favor of intramuscular injections at 2- to 4-hour intervals. Due to the inconvenience of this dosage schedule to both patient and attendants, various methods have been used to prolong the effective concentration in the plasma that results from a single injection of penicillin. These methods decrease the rate either of its absorption from muscle or of its excretion by the kidneys. For decreasing absorption various preparations have been used, for example, a suspension of penicillin in peanut oil and beeswax or a suspension of the procaine salt of penicillin in either oil or water (depository preparations). To decrease excretion by the kidneys, two drugs have been used, carinamide (4) and Benemid (7).

Despite this fact that most dosage schedules of penicillin seem to have been designed with the objective of maintaining the plasma concentrations of this antibiotic at or above levels which are known *in vitro* to inhibit the growth of the invading microorganisms, an examination of the background of this concept shows that it is not supported by established fact (56). In 1944, Fleming and coworkers (40) wrote: "We do not yet know whether it is better to maintain a constant low level of penicillin in the blood, or to have a very high level for a short time after an injection, followed by a period of very low level before the next injection. Clinically both systems have worked excellently. It will take a long series of observations to decide which is the better." In the same year, Tillett and coworkers (95, 96) working with rather crude preparations of penicillin showed that pneumococcal lobar pneumonia could be treated satisfactorily when

¹ Where penicillin is used, it refers either to the original crude preparation or to benzylpenicillin (penicillin G). Other species are appropriately designated.

an interval of 12-16 hours was allowed to elapse between courses of injections of penicillin. At about this time others (21, 64) questioned the necessity of maintaining an effective concentration of penicillin in the blood as is the custom with sulfonamide therapy. In spite of lack of evidence for the concept of a maintained concentration of drug in blood, almost all penicillin therapy is still today based on preparations and dosage schedules which attempt to maintain an effective concentration of drug in plasma. Acceptance of this concept has been so complete that at present the administration of penicillin is directed toward the continued maintenance of a certain concentration of this drug in plasma rather than towards the most efficient therapeutic regimen.

The whole question of the most efficient dosage schedule for penicillin is complicated by the fact that no chemical method has been devised for determining the concentrations of penicillin in plasma and body fluids. Hundreds of reports giving the concentrations of penicillin in plasma, urine, body fluids, and even in tissues have appeared, but what is really being determined is the bacteriostatic power of body fluids and tissues after the administration of penicillin. This is exactly what Moore and Chesney (69) determined after administration of ethylhydrocuprein more than a third of a century ago. However, these authors did not write of the ethylhydrocuprein concentration in serum but used the term "acquired bactericidal property" which described exactly what they were measuring. It is gratifying to find at least one author who writes of the "antibacterial activity of serum" instead of concentration of penicillin (40), and another who states: "No attempt has been made to express the penicillin content of the specimens examined as units of penicillin per ml. of fluid, since at the present time this is not possible without certain assumptions." (63). This latter worker also tested the antibacterial activity of the serum or body fluid before the administration of penicillin and frequently found some activity. This control has been neglected by practically all other workers.

Probably many factors influence the bacteriostatic action of penicillin *in vitro*. One at least has been fairly carefully studied. This is the effect of serum or albumin on the activity of penicillin. It has been found (15, 78, 97) that penicillins G, X and dihydro-F are bound to the extent of fifty to sixty per cent in normal plasma, while penicillin K is at least ninety per cent bound. In using the microbiological method for the assay of penicillin in serum by an ordinary serial dilution of serum, bacteriostasis will occur in the tube where the unbound concentration of drug, not the total concentration, is at a level which just prevents growth. This introduces a very serious error in assays, particularly at low concentrations of drug; and, in fact with low concentrations of penicillin K in serum, there is a correction of more than tenfold (27). Various devices have been used in an attempt to avoid this protein error, but as Davis (20) pointed out, "Even with the best possible technic, the determination in serum of low concentrations of penicillin, especially K, involves considerable error. All of the earlier assays for penicillin, including Eagle's extensive studies on the rate of destruction of the various penicillins in the body, must be reevaluated in the light of the knowledge that an uncorrected assay gives erroneously low values; and since this error is greater at

lower concentrations of drug, the reported rates of disappearance have been greater than the true rates, especially with penicillin K." Tucker (99) has reported that when penicillin is added to spinal fluid (containing only a trace of protein) it cannot be completely recovered by the serial dilution method of assay.

In spite of the fact that determinations of the concentration of penicillin in serum by the microbiological method are questionable, one can from carefully controlled experiments in infected animals arrive at some decision as to the most effective dosage schedule of penicillin. In addition, one can use the unsatisfactory microbiological method to decide if it is necessary to have an effective concentration of penicillin continuously in contact with the microorganism for most effective therapy. A number of such experiments have been reported. In 1946, Jawetz (47) showed that, where measurable blood concentrations persisted for only one hour after injection of penicillin, the depression of bacterial population in a streptococcal infection of mice persisted for at least eight hours. A number of investigators have studied different dosage schedules of penicillin in experimental streptococcal and pneumococcal infections. In some of these studies, the interval between injections was varied, with or without varying the number of injections, but without keeping the total time of treatment constant. The total time of treatment may be more important than the number of injections. Zubrod (109) studied various schedules of penicillin dosage against a hemolytic streptococcal infection of mice and found that cure of the infection could be obtained when penicillin was given at widely spaced intervals. Eight doses at 3-hour intervals were no more and probably were less effective than three doses at 8-hour intervals. This investigator emphasized the importance of a large initial dose regardless of what schedule is adopted thereafter. The advantage consists in the fact that a large initial dose protects mice for the next twenty-four hours, whereas with multiple dose schedules, many mice die before they receive enough drug to bring the infection under control. In this same streptococcal infection White, Baker and Jackson (107) treated mice immediately after infection for a period of twenty-four hours with different dosage schedules of penicillin X. They showed that under these conditions, 1, 2, 4 and 8 doses were equally effective in terms of the total amount of penicillin necessary for the median survival dose for each schedule. Gibson (43) states that the total curative dose of penicillin administered for four days to mice infected with pneumococci was the same whether injections were made at 12- or at 3-hour intervals. However, the number of animals used was small. On the other hand, Eagle, Fleischman and Musselman (29) studied in a pneumococcal infection of mice and in a streptococcal infection of rabbits the effect of varying the intervals between a fixed total number of injections of penicillin. As the interval between the injections was progressively increased, the median curative dose at first decreased to reach a minimum and then increased. Since here the duration of treatment as well as the spacing of injections was varied, the results do not help to clarify the problem of continuous versus intermittent therapy.

Miller *et al.* (68) have studied the dosage schedule of penicillin in an experimental infection in mice with an organism of high penicillin resistance, *Salmonella*

typhosa. They found that a smaller total amount of penicillin was required for protection when treatment was given as a single injection than when 2, 3, 6 or 12 doses were given in a twenty-four hour period. When the time interval between two doses was varied from 2 to 36 hours, it was found that the two dose 2-hour schedule required even a smaller total amount of penicillin than the single dose.

In addition to these studies on mice, we have two extensive and carefully controlled studies on pneumococcal infections of rats. In the first study, Schmidt, Walley and Larson (85) infected rats intraperitoneally with pneumococci and initiated therapy with crystalline penicillin four hours later, at a time when bacteremia ranged from 2000 to 80,000 organisms per cc. The data showed that cure of the infection can be accomplished by the administration of penicillin at either frequently spaced or widely spaced intervals. With treatment of brief duration, two doses of penicillin administered 2 or 4 hours apart were more effective than a single dose or two doses separated by an 8-hour interval, the two doses at a 4-hour interval being the most effective. When a constant period of treatment (96 hours) was used, doses administered at 2-, 4- and 8-hour intervals were equally effective and were more effective than doses at twelve or twenty-four hour intervals. It was also found that procaine penicillin administered at 24-hour intervals was more effective than aqueous penicillin given at 12- or 24-hour intervals but less effective than the aqueous penicillin given on a 2-, 4-, or 8-hour schedule. Measurements were made of the concentration of "penicillin" (bacteriostatic substances) present in plasma after equally effective doses of penicillin on the various dosage schedules. These measurements showed that these equally effective doses maintained inhibitory concentrations of "penicillin" in plasma for only a small fraction of the dosage interval. In the second study, Schmidt and Walley (84) used the same pneumococcus to produce a lobar pneumonia in rats. Here, the animal infection resembles human infections more closely than the fulminating peritonitis usually used. The duration of treatment and dosage schedules were the same as in the first study. This study is of importance in showing that intermittent administration of penicillin, where long periods elapse without a bacteriostatic concentration in plasma, is more effective or takes less total penicillin than in schedules with more frequent dosage. As, in the opinion of the reviewer, this is by far the best and most important study on the dosage schedule of penicillin, the conclusions of the authors are given. "The results of the studies outlined above have shown (1) that the effectiveness of sodium penicillin G in the treatment of established lobar pneumonia is determined by the frequency with which the drug is administered; (2) that by all criteria, sodium penicillin is considerably more effective when administered at 8-hour intervals than when given in either more frequent or more widely spaced doses; (3) that with this most effective dosage schedule, bacteriostatic concentrations of 'penicillin' are maintained in plasma for only a small fraction of the treatment interval; (4) that with respect to either individual or total treatment periods, there appears to be no direct relation between the effectiveness of a regimen and the length of time during which the plasma contains bacteriostatic concentrations of 'penicillin'; (5) that the effectiveness of a dosage schedule appears to be a product of both the height

of the concentration of 'penicillin' in plasma and the duration of detectable concentrations; and (6) that the most effective schedule for administering sodium penicillin G is in every respect superior to the 24-hour administration of the allegedly long acting procaine penicillin."

Although the above findings show that to some extent the effectiveness of penicillin therapy is related to the frequency of treatment, they offer no support for the general assumption that the effectiveness of therapy is dependent upon the continued maintenance of inhibitory concentrations of drug in plasma. Rather, the experimental results indicate that optimal therapeutic effects may be achieved by administration of aqueous penicillin at fairly widely spaced intervals and in fact there is a definite suggestion that intermittent therapy with aqueous penicillin may be more effective than frequent dosage with aqueous penicillin or the use of depositary preparations.

Reports dealing with human infections have proved the efficacy of penicillin therapy which does not maintain an effective concentration in plasma. In pneumococcal pneumonia administration of aqueous penicillin once or twice a day is apparently just as effective as administration at frequent intervals (101, 74, 45, 105). In surgical infections, intermittent administration appears to be satisfactory (1). Streptococcal sore throat has been treated successfully with an intermittent dosage schedule. There were much fewer patients with significant antistreptolysin response in a group on a three hour than in one on a twenty-four hour dosage schedule (49). Just what is the significance of this finding is not clear. Also, patients with acute septic infections of the skin and subcutaneous tissues, mainly staphylococcal, responded as well to aqueous penicillin twice daily as to the injection of procaine penicillin (44). Other authors (98) state that discontinuous penicillin provides entirely satisfactory treatment for pneumonia and the great majority of penicillin-susceptible infections. However, they believe that such discontinuous therapy will be inadequate in the treatment of serious staphylococcal infections, bacterial endocarditis, and certain other rarely encountered infections which require large amounts of penicillin. No proof for this belief in the inadequacy of discontinuous therapy is given.

A different point of view in regard to the dosage schedule of penicillin has been proposed in a long series of papers by Eagle and coworkers. At first, Eagle appeared to accept completely the current idea that it is necessary to maintain, day and night, an effective concentration of penicillin in the plasma for effective therapy. In addition, he believed that *in vitro* results on the sensitivity of parasites to the drug could be transferred without modification to infections in man, as the chemotherapeutic effect of penicillin was a bactericidal one (27). However, evidence had already been given that the body defense mechanisms play a role in the cure of infections with penicillin. McLeod and Stone (52) produced infections in mice with types I and III pneumococcus, which had the same virulence for mice and the same sensitivity to penicillin *in vitro*. Infection with type I was much more easily cured by penicillin than infection with type III. However, Eagle, although he has modified his point of view, still appears to maintain that most effective therapy can be obtained by maintaining a continuous bactericidal

concentration of penicillin in plasma. We can quote several statements from his numerous papers to show this. In 1948, he writes "It is a reasonable surmise that the concentration of penicillin which is maximally effective *in vitro* indicates the approximate level which should be maintained at the focus of infection *in vivo* in order to kill the largest number of organisms in the shortest possible time" (27). In 1950, he writes "A schedule of treatment which provides the maximally effective bactericidal concentration at the focus of infection continuously should therefore be more rapidly effective than a discontinuous schedule" (29). And again, after accepting the idea that the host defense mechanisms do play a role in the action of penicillin and that, as shown by Parker and Marsh (72), and Parker and Luse (71), bacteria exposed to penicillin *in vitro* did not immediately resume multiplication when the drug was removed, he writes, "Although penicillin can therefore be administered on an intermittent schedule without necessarily maintaining its concentration at an effective level, it is a reasonable presumption that bacteria would die more rapidly under the combined impact of penicillin and the host defenses than under the latter alone, and that a maintained bactericidal level would therefore be more rapidly effective than a schedule of treatment supplying that bactericidal concentration only intermittently" (28). In other papers are given tables to show how often a given dose of penicillin must be repeated or what dose must be administered at fixed intervals in order to maintain the concentration of drug in plasma above a certain level (27, 100).

Eagle brings into consideration the fact that the concentration of drug in the plasma does not represent the concentration at the focus of infection. He states, "Due consideration must be given to the fact that most of the bacteria are usually not in the blood stream but in the tissues, and that the plasma level is not necessarily a measure of the actual concentration at the focus of infection" (26). Then another concept is introduced. In discussing the discrepancies of different workers as to whether or not a maintained concentration of penicillin in plasma is necessary for most effective therapy, he writes, "Additional data will be presented herein which are believed to reconcile some of the apparent discrepancies and to reinforce the thesis that the major determinant factor in the therapeutic activity in penicillin is the aggregate time, not necessarily continuous, for which the drug remains at bactericidal levels" (29).

In regard to the first of these concepts that the bacteria are not in the blood stream but in the tissues and that it is the concentration of penicillin in the tissues which is important, the following may be noted. In bacterial infections, the bacteria are not in tissues but in extracellular fluid with the exception of those present in the leucocytes and macrophages. It is, therefore, the concentration of penicillin in extracellular fluid and not that in tissue cells which is important in combating infection. It is also certain that the concentration of free penicillin (that not bound to the plasma albumin) which is the effective agent is roughly the same in plasma and extracellular fluid after equilibrium has been attained. Equilibrium is probably attained or nearly attained very rapidly. After an intravenous injection there is an abrupt fall in the concentration in the plasma for the first ten minutes or so, due presumably to mixing and diffusion into extracellular

space. After this there is a sharp change in the slope of the curve (40). Richardson *et al.* (78) found the apparent volume of distribution of penicillin to be 20 per cent, that is, mainly distributed in extracellular fluid. Determinations of the concentrations of penicillin in plasma and in extracellular fluids (edema, synovial, ascitic and pericardial fluids) are consistent with the concentration of free penicillin being the same as that in plasma, but the inadequacy of analytical methods for estimating the drug prevents drawing definite conclusions.

The above considerations in regard to the penetration of penicillin into extracellular fluids and the rapidity of the attainment of equilibrium may not apply in certain instances. The slow passage of this drug across the "blood-brain barrier" into the cerebrospinal fluid and the possibly slow attainment of equilibrium in normally avascular areas (*e.g.*, vegetations on heart valves) or areas rendered avascular by disease processes (*e.g.*, lung in Friedländer's pneumonia) may be mentioned. Here, it would appear that high peak concentrations in plasma as given by intermittent as opposed to continuous therapy might increase the rate of penetration of penicillin to such regions and hence be more effective. In addition, it is well recognized that cultures of bacteria are composed of both actively growing and resting organisms. On the basis of this observation and the knowledge that penicillin is most effective against rapidly growing organisms, Bigger (5) has maintained that discontinuous administration of penicillin should be more effective than continuous treatment.

The evidence that the concentration of penicillin varies widely in different organs and tissues from the concentration in the plasma (75, 92, 70, 17, 19) is unsatisfactory. As stated above, the microbiological method is full of pitfalls and when applied to tissues is probably much less reliable than when used with plasma or serum. Indeed, in the only study in which I can find recoveries done with tissues, these were as low as 35 per cent, and no data are given to indicate variability (78). No critical analytical chemist would think of trusting data obtained by such a method. The tissue distribution of penicillin must await the devising of an accurate, specific chemical method for its estimation.

The second concept, that the therapeutic activity of penicillin is proportional to the total time, not necessarily continuous, that an effective concentration is maintained, would imply that continuous administration of the drug is more effective in the sense of producing more rapid cure and requiring less total drug. This is directly opposed to all the evidence obtained with varying dosage schedules in infections in experimental animals. Omitting consideration of the large possible errors in the figures used for the calculation of the "total penicillin time," the "relative constancy of this time factor in a given infection" is found actually to have a variation of at least four-fold.

In experimental infections of rabbits with syphilis, studies on dosage schedules were all carried out very early with crude penicillin of varying composition. In one study, it was found that the length of treatment appeared more important to effect a cure than the dosage of the drug. A large single injection was not effective and sixteen injections at hourly intervals were much less effective than thirty-two injections given twice daily at eight-hour intervals (14). The apparent con-

clusions of a second study (31) are as follows: where the interval between injection was fixed and the number of injections varied, the smallest total dose of penicillin necessary to cure was that associated with the largest number of injections or the maximal duration of treatment; where the duration of treatment was constant and the interval between injections varied, it appears that the greater the number of injections, the greater the efficacy of penicillin; where the total number of injections was held constant, the curative dose depended on the interval between injections, doses at intervals of 1 to 2 hours being less effective than doses given twice daily or daily. In a third study (32), "experiments were undertaken to establish the fact that a single injection of penicillin in oil and beeswax, resulting in a prolonged low blood level, was more effective therapeutically than a single injection of the aqueous solution, and just as effective as multiple small injections. . . ." It was found that when injections were made daily for four days, penicillin in oil and beeswax was more effective than aqueous penicillin, and that two daily injections of penicillin in oil and beeswax for 8 days was more effective than a single injection or four daily injections.

In all three of the above studies amorphous crude penicillin of unknown composition (and of varying activity per unit in rabbit syphilis) was used.² In the first study, preparations prepared by six different manufacturers were used; in the second, eighteen different lots from nine different sources, which were used, varied in activity from 130 to 1025 units per milligram; and in the third, nine different lots, four from commercial sources and the rest homemade, were employed. The very small number of animals used makes it impossible to detect differences in activity between the different lots of penicillin. In addition, all these studies attempted to decide too many issues with too few animals; there is a lack of any attempt to estimate the standard errors, which in many cases cannot even be calculated. Therefore, one must accept with reservations any conclusions on the most effective dosage schedule in rabbit syphilis.

There are no data which led to unequivocal conclusions as to the most efficient method of using penicillin in the treatment of human syphilis. It has been assumed that the best method of treatment is one which attempts to maintain a more or less constant and effective concentration of penicillin in the plasma. Due to this preconception about therapy, little is known about the curative effect of widely spaced doses of aqueous penicillin. The conduct of satisfactory, well-controlled experiments in the treatment of human syphilis is beset with many difficulties. Among these are the difficulty of distinguishing relapse and reinfection, the long period of follow-up necessary with its inevitable loss of patients from the study, and the criteria of failure used.

The reports of the Central Statistical Unit in a Nation-wide Study of the

² The potency of penicillin in units is determined by its *in vitro* activity against a strain of *S. aureus*. Different species (G, F, X and K) show different activities; G possesses a potency of 1667 units per milligram; F, of 1550 units; X, of 900 units; K, of 2300 units. Compared to G, penicillins F, X, and K have much less activity in rabbit syphilis (3, 102). For further discussion of amorphous penicillin and its changing character see the Report of Committee on Medical Research, The United States Public Health Service and the Food and Drug Administration (16).

Treatment of Early Syphilis (67, 66, 79) give some information on the efficacy of various dosage schedules of penicillin. Cumulative failure rates were used as a criterion of efficacy. In the early studies with amorphous crude penicillin, no significant differences in failure rates could be detected when any total dose from 1.2 to 4.8 million units was given within four days to two weeks at intervals of 2 to 6 hours. In a later more satisfactorily planned study, it was found that using crystalline penicillin, no differences were found with four schedules involving a total dosage of 2.4 and 4.8 million units for 7.5 days and intervals of 2 and 3 hours between doses (65). It is difficult to understand the finding that crystalline penicillin G given in total dosage of 2.4 and 4.8 million units over 7.5 days gives results superior to comparable dosage schedules of amorphous crude penicillin (79). In view of the fact that no difference was found in treatment using a total dosage of 1.2, 2.4, and 4.8 million units of amorphous penicillin, it would appear that the unitage of the amorphous penicillin used at this time did not at all correlate with its antisyphilitic activity.

In regard to the effect of varying the duration of treatment, the Central Statistical Unit found no difference in regimes of 4, 7.5 and 15 days. In addition, it has been found that very large doses of penicillin given over a twenty-four hour period are grossly inadequate for the treatment of syphilis (73).

Due to practical considerations, the various types of depositary penicillin have almost replaced aqueous penicillin in the treatment of syphilis. The desire is apparently to use the one which gives a measurable concentration of antibacterial substance in the plasma for the longest time; at present, procaine penicillin in oil with aluminum monostearate appears to be the most used preparation.

From the above discussion, it appears to the reviewer that it is impossible to state that the most effective dosage schedule of penicillin for the treatment of syphilis requires a maintained concentration in plasma; fairly widely spaced injections of aqueous penicillin may be just as effective.

The situation in regard to the dosage schedule of streptomycin in bacterial infections was quite similar to that with penicillin. Although the concentration of drug in blood is maintained much longer from an injection of streptomycin than from one of penicillin (54), the original dosage schedule of streptomycin required injection of drug every three or four hours. Experiments, however, showed that just as effective therapy is obtained when the drug is given infrequently as when it is given every few hours around the clock. Thus, in a *K. pneumoniae* infection of mice, Zubrod (110) found that the total daily dose of streptomycin administered, rather than the number of fractions into which the total dose is divided, conditioned the therapeutic response. In experimental tuberculosis in the guinea pig, it has been shown 1) that the effectiveness of the drug depends on the length of treatment (91), and 2) that a dose once a day or even at less frequent intervals is just as effective as a dosage schedule requiring administration at four- or six-hour intervals (36, 35).

In human tubercular infection, a dosage schedule of streptomycin which does not maintain a more or less constant concentration of drug in the blood is effec-

tive. Thus, one dose a day (6) and even doses only twice a week (77) are considered effective.

No data are available on the most effective dosage schedule of para-aminosalicylic acid in tuberculosis. It has been given in frequent divided doses mainly in order to give as much drug as possible without disturbing gastrointestinal symptoms.

In regard to the most effective dosage schedules of aureomycin, terramycin and chloramphenicol, no statement of any kind can be made. There is a complete lack of data bearing on the problem. With aureomycin, a limited study of bacterial infections in mice showed that a single large injection of drug immediately after infection was less effective than a dosage schedule of three times daily for three days (11). This, however, has little bearing on the problem of continuous versus intermittent administration. In clinical use, all three of these drugs are usually administered every four or six hours, possibly the best spacing to maintain effective concentrations in blood and to prevent or minimize gastrointestinal irritation.

Although Moore and Chesney were concerned with the need of the most satisfactory dosage schedule of ethylhydrocuprein over a third of a century ago, today little attention is given in the clinical use of new chemotherapeutic agents to finding the most effective and satisfactory method of administration. In the case of the sulfonamides, the dosage schedule used by Moore and Chesney for ethylhydrocuprein was shown to be the most effective and therapy was actually based upon concentrations of drug in blood rather than dosage by mouth. In the case of the antimalarial drugs, the dosage schedules, while to some extent a carry over from the sulfonamides, are probably correct. With penicillin, there is little relation between the clinical dosage schedule in use and the facts obtained from experimental therapy. With the newer antibiotics even experiments on dosage schedules in infected animals are lacking.

REFERENCES

1. ALTEMEIER, W. A.: Penicillin therapy with prolonged interval dosage schedules. *Transactions American Surgical Association*, **66**: 388-394, 1948.
2. ALVING, A. S., CRAIG, B., JR., WHORTON, C. M., JONES, R., JR. AND EICHELBERGER, L.: Procedures used at Stateville Penitentiary for the testing of potential antimalarial agents. *J. Clin. Investigation*, **27** (Pt. 2), 2-5, 1948.
3. ARNOLD, R. C., BOAK, R. A., CARPENTER, C. M., CHESNEY, A. M., FLEMING, W. L., GUEFF, B., MAHONEY, J. F. AND ROSAHN, F. D.: A joint report on a cooperative investigation of the efficacy of species of penicillin in the treatment of experimental syphilis. *Am. J. Syph., Gonorr. & Ven. Dis.*, **31**: 469-475, 1947.
4. BEYER, K. H., MILLER, A. K., RUSSO, H. F., PATCH, E. A. AND VERWEY, W. F.: The inhibitory effect of Caronamide on the renal elimination of penicillin. *Am. J. Physiol.*, **149**: 355-368, 1947.
5. BIGGER, J. W.: Treatment of staphylococcal infections with penicillin by intermittent sterilization. *Lancet*, **247**: 497-500, 1944.
6. BIGNALL, J. R., CLEGG, J. W., CROFTON, J. W., SMITH, R. J. D., HOLY, H. D., MITCHELSON, D. A. AND ARMITAGE, P.: Intermittent dosage in the treatment of pulmonary tuberculosis with streptomycin. A Report to the Streptomycin in Tuberculosis Committee of the Medical Research Council. *Brit. M. J.*, **1**: 1224-1230, 1950.
7. BOGER, W. P., BEATTY, J. O., FITTS, F. W. AND FLIPPIN, H. F.: The influence of a new benzoic acid derivative on the metabolism of para-amino-salicylic acid (PAS) and penicillin. *Ann. Int. Med.*, **33**: 18-31, 1950.
8. BRATTON, A. C., JR.: Continuous intravenous chemotherapy of plasmodium falciparum infection in ducks. *J. Pharmacol. & Exper. Therap.*, **85**: 103-110, 1945.
9. BRATTON, A. C. AND MARSHALL, E. K., JR.: A new coupling component for sulfanilamide determination. *J. Biol. Chem.*, **128**: 537-550, 1939.

10. BRYANT, J.: Heavy atebirin dosage in the treatment of malaria. *East African M. J.*, **15**: 295-301, 1942.
11. BRYER, M. S., SCHOENBACH, E. B., BLISS, E. A. AND CHANDLER, C. A.: Treatment of experimental infections with aureomycin. *Ann. New York Acad. Sci.*, **51**: 254-266, 1948.
12. BUTTLE, G. A. H., GRAY, W. H. AND STEPHENSON, D.: Protection of mice against streptococcal and other infections by p-aminobenzenesulphonamide and related substances. *Lancet*, **230**: 1286-1290, 1936.
13. CANFIELD, G. AND SCHMIDT, L. H.: Personal Communication.
14. CARPENTER, C. M., BOAK, R. A. AND JACOBS, L. M.: The treatment of experimental syphilis with penicillin. *New York State J. Med.*, **46**: 2150-2153, 1946.
15. CHOW, B. F. AND MCKEE, C. M.: Interaction between crystalline penicillin and human plasma proteins. *Science*, **101**: 67-68, 1945.
16. Committee on Medical Research, The United States Public Health Service and the Food and Drug Administration. The changing character of commercial penicillin. A joint statement. *J. Am. M. A.*, **131**: 271-275, 1946.
17. COOKE, J. V. AND GOLDRING, D.: The concentration of penicillin in various body fluids during penicillin therapy. *J. Am. M. A.*, **127**: 80-87, 1945.
18. COOPER, W. C.: Summary of antimalarial drugs. *Pub. Health Rep.*, **64**: 717-732, 1949.
19. CUTTING, W. C., LUDWIG, F. P., FIESE, M., ELLIOTT, H. W. AND FIELD, J. H.: Distribution and fate of penicillin in the body. *J. Pharmacol. & Exper. Therap.*, **85**: 36-41, 1945.
20. DAVIS, B. D.: The binding of chemotherapeutic agents to proteins and its effect on their distribution and activity. In "Evaluation of Chemotherapeutic Agents" (Symposia of the Section on Microbiology, The New York Academy of Medicine). Edited by C. M. MacLeod, pp. 44-64. Columbia Univ. Press, New York, 1949.
21. DAWSON, M. H. AND HOBBS, G. L.: The clinical use of penicillin. Observations in one hundred cases. *J. Am. M. A.*, **124**: 611-622, 1944.
22. DEARBORN, E. H.: A comparison of one dose per day with six doses per day of quinine in the suppression of lophure malaria in the duck. *J. Pharmacol. & Exper. Therap.*, **94**: 178-181, 1948.
23. DEARBORN, E. H.: The distribution of quinacrine in dogs and in rabbits. *J. Pharmacol. & Exper. Therap.*, **91**: 174-177, 1947.
24. DI CRISTINA, G. AND CARONIA, G.: Sulla terapia della leishmaniosi interna. *La Pediatria*, **23**: 81-92, 1915.
25. DUNCAN, G. G.: Quinacrine hydrochloride as a malaria-suppressive agent for combat troops. *War Med.*, **8**: 305-318, 1945.
26. EAGLE, H.: Host, drug and parasite factors that modify the therapeutic activity of penicillin. In *Advances in Internal Medicine*, **3**: 105-149, 1949.
27. EAGLE, H.: Speculations as to the therapeutic significance of the penicillin blood level. *Ann. Int. Med.*, **20**: 260-278, 1948.
28. EAGLE, H., FLEISCHMAN, R. AND MUESELMAN, A. D.: The bactericidal action of penicillin *in vivo*: The participation of the host, and the slow recovery of the surviving organisms. *Ann. Int. Med.*, **23**: 544-571, 1950.
29. EAGLE, H., FLEISCHMAN, R. AND MUESELMAN, A. D.: Effect of schedule of administration on the therapeutic efficacy of penicillin. *Am. J. Med.*, **9**: 280-299, 1950.
30. EAGLE, H. AND HOGAN, R. B.: An experimental evaluation of intensive methods for the treatment of early syphilis. II. Therapeutic efficacy and margin of safety. *Ven. Dis. Inform.*, **24**: 69-79, 1943.
31. EAGLE, H., MAGNUSON, H. J. AND FLEISCHMAN, R.: The effect of the method of administration on the therapeutic efficacy of sodium penicillin in experimental syphilis. *Bull. Johns Hopkins Hosp.*, **79**: 168-189, 1946.
32. EAGLE, H., MAGNUSON, H. J. AND FLEISCHMAN, R.: Observations on the therapeutic efficacy in experimental syphilis of calcium penicillin in oil and beeswax and their bearing on its use in man. *Am. J. Syph., Gonorr. & Ven. Dis.*, **31**: 246-256, 1947.
33. ELLERBROOK, L. D., LIPPINCOTT, S. W., CATENO, C. F., GORDON, H. H. AND MARBLE, A.: Plasma quinacrine concentration in treatment of plasmodium vivax malaria acquired in the South Pacific. *Arch. Int. Med.*, **76**: 352-357, 1945.
34. FEINSTONE, W. H., BLISS, E. A., OTT, E. AND LONG, P. H.: Observations concerning the toxicity, absorption and therapeutic effect of sulphanilamide and certain related organic sulphur-containing compounds in experimental infections in mice. *Bull. Johns Hopkins Hosp.*, **62**: 565-592, 1938.
35. FELDMAN, W. H.: The chemotherapy of tuberculosis—including the use of streptomycin. *Lecture No. 3. The effect on tuberculosis of antagonistic substances of microbial origin with particular reference to streptomycin.* (The Harben Lectures, 1946.) *J. Roy. Inst. Pub. Health & Hyg.*, **9**: 343-363, 1948.
36. FELDMAN, W. H., HINSHAW, H. C. AND KARLSON, A. C.: Frequency of administration of streptomycin. Its influence on results of treatment of tuberculosis in guinea pigs. *Am. Rev. Tuberc.*, **55**: 435-443, 1947.
37. FINDLAY, G. M.: Recent Advances in Chemotherapy. 3rd ed., **1**: 289-302, 1950.
38. FINDLAY, G. M.: Recent Advances in Chemotherapy. 3rd ed., **1**: 404-408, 1950.
39. FINDLAY, G. M., MARKSON, J. L. AND HOLDEN, J. R.: Investigations in the chemotherapy of malaria in West Africa. I. Treatment with quinine and mepacrine. *Ann. Trop. Med. & Parasit.*, **38**: 139-146, 1944.
40. FLEMING, A., YOUNG, M. Y., SURET, J. AND ROWE, A. J. E.: Penicillin content of blood serum after various doses of penicillin by various routes. *Lancet*, **247**: 621-624, 1944.
41. FULLER, A. T.: Is p-Aminobenzenesulphonamide the active agent in prontosil therapy? *Lancet*, **232**: 194-198, 1937.
42. FULTON, J. D. AND YORKE, W.: Studies in chemotherapy, XXXIV. Comparison of the results obtained by different methods of administration of drugs in trypanosomal infections of mice. *Ann. Trop. Med. & Parasit.*, **37**: 80-95, 1943.
43. GIBSON, C. D.: Comparative effectiveness of two penicillin treatment schedules in pneumococcal infections of mice. *Proc. Soc. Exper. Biol. & Med.*, **67**: 278-280, 1948.

41. GRIFFITHS, E., JONES, P. F., SHOOTER, R. A. AND HEADY, J. A.: The comparative merits of sodium and procaine penicillin given infrequently. *Brit. M. J.*, **2**: 958-960, 1949.
42. HAMBURGER, M., BERMAN, J. R., THOMPSON, R. T. AND BLANKENHORN, M. A.: Treatment of pneumococcal pneumonia by penicillin in aqueous solution at long intervals. *J. Lab. & Clin. Med.*, **34**: 59-66, 1949.
43. HYMAN, H. T., CHARGIN, L., RICE, J. L. AND LEIFER, W.: Massive dose chemotherapy of early syphilis by intravenous drip method. *J. Am. M. A.*, **113**: 1206-1214, 1939.
44. JAWETZ, E.: Dynamics of the action of penicillin in experimental animals. Observations on mice. *Arch. Int. Med.*, **77**: 1-15, 1946.
45. LITCHFIELD, J. T., WHITE, H. J. AND MARSHALL, E. K., JR.: The comparative therapeutic activity of sulfanilamide, sulfapyridine, sulfathiazole and diaminosulfone in type I pneumococcus infections in mice. *J. Pharmacol. & Exper. Therap.*, **69**: 166-170, 1940.
46. LOSE, J. P. AND KILBOURNE, E. D.: Penicillin treatment of streptococcal pharyngitis. *Ann. Int. Med.*, **29**: 698-714, 1948.
47. LONG, P. H. AND BLISS, E. A.: The use of para-amino benzene sulphonamide (sulfanilamide) or its derivatives in the treatment of infections due to beta hemolytic streptococci, pneumococci and meningococci. *South. M. J.*, **30**: 479-487, 1937.
48. MACGILCHRIST, A. C.: The relative therapeutic value in malaria of the cinchona alkaloids—quinine, cinchonine, quinidine, cinchonidine and quinoidine, and the two derivatives—hydroquinine and ethylhydrocupreine. *Indian J. M. Research*, **3**: 1-89, 1915-16.
49. MACLEOD, C. M. AND STONE, E. R.: Differences in the nature and antibacterial action of the sulfonamides and penicillin and their relation to therapy. *Bull. New York Acad. Med.*, **21** (2nd ed.), 375-388, 1945.
50. MARKE, L. H.: Chemotherapeutische Versuche bei Vogel-malaria. *Berliner Klin. Wchnschr.*, **51**: 1886-1888, 1914.
51. MARSHALL, E. K., JR.: The absorption, distribution and excretion of streptomycin. *J. Pharmacol. & Exper. Therap.*, **92**: 43-48, 1948.
52. MARSHALL, E. K., JR.: Determination of sulfanilamide in blood and urine. *J. Biol. Chem.*, **122**: 263-273, 1937.
53. MARSHALL, E. K., JR.: The dosage schedule of penicillin in bacterial infections. *Bull. Johns Hopkins Hosp.*, **82**: 403-407, 1948.
54. MARSHALL, E. K., JR.: The significance of the drug concentration in the blood as applied to chemotherapy. In "Evaluation of Chemotherapeutic Agents" (Symposia of the Section on Microbiology, the New York Academy of Medicine). Edited by C. M. MacLeod, pp. 3-24. Columbia Univ. Press, New York, 1949.
55. MARSHALL, E. K., JR. AND DEARBORN, E. H.: Comparison of drug-diet therapy with single daily oral dosage in avian malaria. *J. Pharmacol. & Exper. Therap.*, **88**: 187-189, 1946.
56. MARSHALL, E. K., JR. AND DEARBORN, E. H.: The relation of the plasma concentration of quinacrine to its anti-malarial activity. *J. Pharmacol. & Exper. Therap.*, **88**: 142-153, 1946.
57. MARSHALL, E. K., JR., EMERSON, K. AND CUTTING, W. C.: Para-aminobenzenesulfonamide, absorption and excretion: method of determination in urine and blood. *J. Am. M. A.*, **100**: 933-957, 1937.
58. MARSHALL, E. K., JR., LITCHFIELD, J. T. AND WHITE, H. J.: The comparative therapeutic activity of sulfanilamide, sulfapyridine and diaminosulfone in streptococcus infections in mice. *J. Pharmacol. & Exper. Therap.*, **69**: 89-102, 1940.
59. MARSHALL, E. K., JR., LITCHFIELD, J. T. AND WHITE, H. J.: Sulfonamide therapy of malaria in ducks. *J. Pharmacol. & Exper. Therap.*, **75**: 89-104, 1942.
60. McADAM, I. W. J., DEGUDE, J. P., CHALLINOR, S. W. AND McCALL, A.: Penicillin treatment of serous-cavity infections. *Lancet*, **249**: 843-848, 1945.
61. McDERMOTT, W., BENNETT, M. AND DuBOIS, R.: Time-dose relationships of penicillin therapy. III. Regimens used in early syphilis. *Am. J. Syph., Gonorr. & Ven. Dis.*, **29**: 345-352, 1945.
62. MERRELL, M.: Report from Central Statistical Unit on Comparative Failure Rates for Early Syphilis Treated with Penicillin. In A Symposium on Current Progress in the Study of Venereal Diseases published by the Division of Venereal Disease, U. S. Public Health Service, pp. 40-45, 1949.
63. MERRELL, M.: Report of the Central Statistical Unit. I. Schedules involving amorphous penicillin in aqueous solution. In "Recent Advances in the Study of Venereal Diseases." A Symposium published by the Venereal Disease Education Institute in cooperation with the U. S. Public Health Service, pp. 88-95, 1948.
64. MERRELL, M.: Results of the Nation-wide Study on Treatment of Early Syphilis (A Report of the Central Statistical Unit). Presented at a Symposium entitled "Recent Advances in the Investigation of Venereal Diseases held under the auspices of the Syphilis Study Section, Research Grants Division, National Institute of Health," U. S. Public Health Service, April 17, 1947.
65. MILLER, A. K., WILMER, D. L. AND VERWEY, W. F.: Effect of penicillin dosage schedule on treatment of experimental typhoid infections in mice. *Proc. Soc. Exper. Biol. & Med.*, **74**: 62-64, 1950.
66. MOORE, H. F. AND CHENEY, A. M.: A study of ethylhydrocupreine (optochin) in the treatment of acute lobar pneumonia. *Arch. Int. Med.*, **19**: 611-682, 1917.
67. ORY, E. M., MEADS, M., BROWN, B., WILCOX, C. AND FINLAND, M.: Penicillin levels in serum and in some body fluids during systemic and local therapy. *J. Lab. & Clin. Med.*, **30**: 809-829, 1945.
68. PARKER, R. F. AND LUSE, S.: The action of penicillin on staphylococcus: Further observations on the effect of a short exposure. *J. Bact.*, **56**: 75-81, 1948.
69. PARKER, R. F. AND MARSH, H. C.: The action of penicillin on staphylococcus. *J. Bact.*, **51**: 181-186, 1946.
70. PETERS, E. E. AND BARTON, R. L.: The massive intravenous penicillin therapy in early syphilis. *Am. J. Syph., Gonorr. & Ven. Dis.*, **31**: 322-332, 1947.

74. PRICE, A. H.: Aqueous penicillin therapy for pneumococcal pneumonia: Injections at twelve hour intervals. *J. Am. M. A.*, **138**: 292-293, 1948.
75. RAMMELKAMP, C. H. and KEEFER, C. S.: The absorption, excretion, and distribution of penicillin. *J. Clin. Investigation*, **22**: 425-437, 1943.
76. RAMSDEN, W., LIPKINS, I. J. and WHITLEY, E.: On quinine in animal tissues and liquids, with methods for its estimation. *Ann. Trop. Med. & Parasit.*, **12**: 223-258, 1918-19.
77. Report to Council on Pharmacy and Chemistry. Chemotherapy of tuberculosis in man. Submitted by Arthur M. Walker, Secretary of the Veterans Administration Committee on Streptomycin. *J. Am. M. A.*, **147**: 253-258, 1951.
78. RICHARDSON, A. P., MILLER, I., SCHUMACHER, C., JAMBOR, W., PANSY, F. and LAPFEDER, D.: Physiological disposition of penicillin G and K in dogs. *Proc. Soc. Exper. Biol. & Med.*, **63**: 514-519, 1946.
79. RIDER, R. V.: Reports of the Central Statistical Unit. II. Amorphous penicillin versus crystalline penicillin G. III. Aqueous penicillin versus penicillin-oil-beeswax. *IN "Recent Advances in the Study of Venereal Diseases."* A Symposium published by the Venereal Disease Education Institute in cooperation with the U. S. Public Health Service, pp. 99-106, 1948.
80. ROEHL, W.: Die Wirkung des Plasmodiums auf die Vogelmalária. *Arch. f. Schiffs. u. Tropen. Hyg. Beihefte*, **30**: 311-318, 1926.
81. SCHMIDT, L. H.: Use of sulfanilamide in the treatment of type XIV pneumococcus infections in mice. *Proc. Soc. Exper. Biol. & Med.*, **37**: 205-206, 1937.
82. SCHMIDT, L. H. and HILLES, C.: The therapeutic properties of sulfanilamide in experimental pneumococcal infections. *J. Infect. Dis.*, **65**: 273-284, 1939.
83. SCHMIDT, L. H., SQUIRES, W. L. and SPIES, M.: Personal Communication.
84. SCHMIDT, L. H. and WALLEY, A.: The influence of the dosage regimen on the therapeutic effectiveness of penicillin G in experimental lobar pneumonia. *J. Pharmacol. & Exper. Therap.*, **104**: 479-488, 1951.
85. SCHMIDT, L. H., WALLEY, A. and LARSON, R. D.: The influence of the dosage regimen on the therapeutic activity of penicillin G. *J. Pharmacol. & Exper. Therap.*, **96**: 258-268, 1949.
86. SCHOENBACH, E. B. and GREENSPAN, E. M.: The pharmacology, mode of action and therapeutic potentialities of stilbamidine, pentamidine, propamidine and other aromatic diamidines. A Review. *Medicine*, **27**: 327-377, 1948.
87. SHANNON, J. A.: Progress in antimalarial therapy. *J. Am. Pharm. A. (Pharm. Ed.)*, **7**: 162-157, 1946.
88. SHANNON, J. A.: The study of antimalarials and antimalarial activity in human malaria. *Harvey Lects.*, **41**: 43-89, 1945.
89. SHANNON, J. A., EARLE, D. P., JR., BERLINER, R. W. and TAGGART, J. V.: Studies on the chemotherapy of the human malaria. I. Method for the quantitative assay of suppressive antimalarial action in vivax malaria. *J. Clin. Investigation*, **27** (Pt. 2), 66-79, 1948.
90. SHANNON, J. A., EARLE, D. P., JR., BRODIE, B. B., TAGGART, J. V., BERLINER, R. W. and the Resident Staff of the Research Service: The pharmacological basis for the rational use of atabrine in the treatment of malaria. *J. Pharmacol. & Exper. Therap.*, **81**: 307-330, 1944.
91. SMITH, M. I., EMMART, E. W. and McCLOSKEY, W. T.: Streptomycin in experimental guinea pig tuberculosis. *Am. Rev. Tuberc.*, **58**: 112-122, 1948.
92. STRUBLE, G. C. and BELLOW, J. G.: Studies in the distribution of penicillin in the eye. *J. Am. M. A.*, **125**: 685-690, 1944.
93. A Survey of Antimalarial Drugs, 1941-1945. Subsidized by The Office of Scientific Research and Development on Recommendation by the Committee on Medical Research. Ed. by F. Y. Wisclogie, **1**: 1-220, 1946. J. W. Edwards, Ann Arbor, Michigan.
94. TAGGART, J. V., EARLE, D. P., JR., BERLINER, R. W., ZUBROD, C. G., WELCH, W. J., WISE, N. B., SCHROEDER, E. F., LONDON, I. M., and SHANNON, J. A.: Studies on the chemotherapy of the human malaria. III. The physiological disposition and antimalarial activity of the cinchona alkaloids. *J. Clin. Investigation*, **27**: 80-86, 1948.
95. TILLET, W. S., CAMBIE, M. J., and HARRIS, W. H., JR.: The treatment of lobar pneumonia and pneumococcal empyema with penicillin. *Bull. New York Acad. Med.*, **20**: 142-178, 1944.
96. TILLET, W. S., MCCORMACK, J. E. and CAMBIE, M. J.: The treatment of lobar pneumonia with penicillin. *J. Clin. Investigation*, **24**: 589-594, 1945.
97. TOMPKETT, R., SCHULTZ, S. and McDERMOTT, W.: Influence of protein binding on the interpretation of penicillin activity *in vivo*. *Proc. Soc. Exper. Biol. & Med.*, **65**: 163-172, 1947.
98. TOMPKETT, R., TIMPANELLI, A., GOLDSTEIN, O. and McDERMOTT, W.: Discontinuous therapy with penicillin. *J. Am. M. A.*, **139**: 555-559, 1949.
99. TUCKER, H. A.: Effect of human cerebrospinal fluid on the dilution bioassay of penicillins G, X and K. *Am. J. Clin. Path.*, **18**: 737-741, 1948.
100. TUCKER, H. A. and EAGLE, H.: Serum concentrations of penicillin G in man following intramuscular injection in aqueous solution and in peanut oil-beeswax suspension. *Am. J. Med.*, **4**: 343-354, 1948.
101. TEMULY, P. A. and ZUBROD, C.: Pneumococcal pneumonia treated with aqueous penicillin at twelve-hour intervals. *New England J. Med.*, **239**: 1033-1036, 1948.
102. TURNER, T. B., CUMBERLAND, M. C. and LI, H. Y.: Comparative effectiveness of penicillins G, F, K and X in experimental syphilis as determined by a short *in vivo* method. *Am. J. Syph., Gonorr. & Ven. Dis.*, **31**: 476-484, 1947.
103. VIANNA, G.: *Archivos Brasileiros de Medicina*, **11**, 422-428, 1912.

104. WANG, C. W.: Solustibosan in treatment of kala-azar in Chinese hamsters. *Proc. Soc. Exper. Biol. & Med.*, **41**: 152-155, 1951.
105. WEISS, W. AND STEINBERG, I.: Treatment of pneumonia with intramuscular aqueous penicillin once a day. *Am. J. M. Sc. (N.S.)*, **217**: 86-91, 1949.
106. WERNER, A. E. A.: Estimation of sulphanilamide in biological fluids. *Lancet*, **236**: 18-20, 1939.
107. WHITE, H. J., BAKER, M. J. AND JACKSON, E. R.: Therapeutic effectiveness of single and divided doses of penicillin in a streptococcal infection in mice. *Proc. Soc. Exper. Biol. & Med.*, **47**: 199-203, 1948.
108. WHITE, H. J., LITCHFIELD, J. T., AND MARSHALL, E. K., JR.: Quantitative comparisons of the activity of sulfanilamide, sulfapyridine, sulfathiazole and sulfadiazine against *E. coli* *in vivo* and *in vitro*. *J. Pharmacol. & Exper. Therap.*, **73**: 104-118, 1941.
109. ZUBROD, C. G.: Comparative efficiency of single and multiple dosage regimens of the penicillins. *Bull. Johns Hopkins Hosp.*, **81**: 400-410, 1947.
110. ZUBROD, C. G.: Relation of dosage schedule to therapeutic efficiency of streptomycin in the treatment of *K. pneumoniae* infections in mice. *Bull. Johns Hopkins Hosp.*, **82**: 357-365, 1948.

